

# Package ‘InTAD’

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**Type** Package

**Title** Search for correlation between epigenetic signals and gene expression in TADs

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**Description** The package is focused on the detection of correlation between expressed genes and selected epigenomic signals (i.e. enhancers obtained from ChIP-seq data) either within topologically associated domains (TADs) or between chromatin contact loop anchors. Various parameters can be controlled to investigate the influence of external factors and visualization plots are available for each analysis step.

**License** GPL (>=2)

**LazyData** TRUE

**Depends** R (>= 3.5), methods, S4Vectors, IRanges, GenomicRanges, MultiAssayExperiment, SummarizedExperiment, stats

**Imports** BiocGenerics, Biobase, rtracklayer, parallel, graphics, mclust, qvalue, ggplot2, utils, ggpubr

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combineInTAD	<i>Preparation for correlation analysis</i>
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---

## Description

This function combines signals and genes in inside of Topologically Associated Domains (TADs)

## Usage

```
combineInTAD(object, tadGR, selMaxTadOvlp = TRUE, closestGene = TRUE)
```

## Arguments

object	InTADSig object
tadGR	TAD genomic regions
selMaxTadOvlp	If a signal overlaps 2 or more TADs by default only single TAD with max overlap is selected. All overlaps can be included by deactivating this option.
closestGene	By default closest to TAD genes are selected based on TSS location. Deactivate this option to use genes only lying within TAD.

## Details

Each signal is checked if it is lying inside of TAD. Signals out of TADs are ignored. The genomic regions representing gene coordinates are converted to TSS. By default, the closest genes are assigned belonging to TAD. If this option deactivated, only those lying with TAD are collected. Result is a list of signals connected to tables with gene details.

**Value**

Updated InTADSig object containing genes connected to each signal

**Examples**

```
# create sigInTAD object
inTadSig <- newSigInTAD(enhSel, enhSelGR, rpkmCountsSel, txsSel)
# combine signals and genes in TAD
inTadSig <- combineInTAD(inTadSig, tadGR)
```

---

combineWithLoops

*Preparation for correlation analysis via loops*

---

**Description**

This function combines signals and genes based on the usage of loops obtained from HiC data analysis

**Usage**

```
combineWithLoops(object, loopsInitDf, fragmentLength = 0, tssWidth = 2000,
  extSize = 0)
```

**Arguments**

object	InTADSig object
loopsInitDf	Data frame with loops. By default 6-column format ( <i>chr1,start1,end1,chr2,start2,pos2</i> ) is expected.
fragmentLength	In case the input format is 4-column ( <i>chr1,middlePos1, chr2, middlePos2</i> ) fragment length should be provided to extend the corresponding loci for loop start and end positions.
tssWidth	The transcription start site width is used to control overlaps with loop anchor. Default is 2000 base pairs.
extSize	The loop endings can be extended upstream and downstream with provided corresponding increase size in base pairs.

**Details**

The expected input is the loops data.frame applied to find connections of signals to genes. This data.frame could be in two formats: either (*chr1,start1,end1,chr2,start2,end2*) or (*chr1,middlePos1,chr2,middlePos2*) with fragment size.

**Value**

Updated InTADSig object containing genes connected to signals via loops

---

enhSel	<i>Enhancer signals subset detected from medulloblastoma samples</i>
--------	--

---

**Description**

This data.frame contains 65 selected in chr15 normalized enhancers signals subset from 25 medulloblastoma samples.

**Usage**

```
enhSel
```

**Format**

a data.frame instance

**Value**

NULL, but makes available the dataframe

---

enhSelGR	<i>Genomic coordiantes of enhancer signals subet</i>
----------	--

---

**Description**

This GRanges object contains the coordinates of 65 medulloblastoma enhancer signals in chr15 target region

**Usage**

```
enhSelGR
```

**Format**

a GRanges object

**Value**

NULL, but makes available the dataset

---

exprs, InTADSig-method *Gene expression counts table*

---

### Description

This function returns gene expression counts table

### Usage

```
## S4 method for signature 'InTADSig'
exprs(object)
```

### Arguments

object            InTADSig object with signals and genes

### Value

Gene expression table

### Examples

```
inTadSig <- newSigInTAD(enhSel, enhSelGR, rpkmCountsSel, txsSel)
head(exprs(inTadSig))
```

---

filterGeneExpr            *Function to filter gene expression*

---

### Description

This function performs filtering of gene expression counts based on various parameters

### Usage

```
filterGeneExpr(obj, cutVal = 0, geneType = NA, checkExprDistr = FALSE,
  plotExprDistr = FALSE)
```

### Arguments

obj            InTADSig object

cutVal        Exclude genes that have max expression less or equal to this value in all samples.  
Default: 0

geneType     Type of gene to select for filtering i.e. "protein\_coding". Default:NA

checkExprDistr Adjust cutVal based on gene expression distribution

plotExprDistr Perform visualziation of the distribution

**Details**

The function allows to stabilize the functional activity of the genes. By default all not expressed genes are filtered. It is also possible to set type of gene to take into account i.e. "protein\_coding" only. This option requires additional metadata column "transcript\_type". Also, special filtering option based on mclust library allows to analyze distribution of counts and adjust the cut value to exclude low expressed genes.

**Value**

InTADSig object with filtered counts table

**Examples**

```
## perform analysis on test data
inTadSig <- newSigInTAD(enhSel, enhSelGR, rpkmCountsSel, txsSel)
## default filtering
inTadSig <- filterGeneExpr(inTadSig)
## filter based on gene type
inTadSig <- filterGeneExpr(inTadSig, geneType = "protein_coding")
```

---

findCorFromLoops

*Function to perform correlation analysis via loops.*

---

**Description**

This function combines genes and signals using obtained loop connections.

**Usage**

```
findCorFromLoops(object, method = "pearson", adj.pval = FALSE)
```

**Arguments**

object	InTADSig object with signals and genes combined via loops
method	Correlation method: "pearson" (default), "kendall", "spearman"
adj.pval	Perform p-value adjustment and include q-values in result

**Value**

A table with correlation values for signal-gene pairs including correlation p-value and euclidian distance.

---

findCorrelation	<i>Function to perform correlation analysis in TADs</i>
-----------------	---

---

**Description**

This function combines genes and signals in inside of TADs

**Usage**

```
findCorrelation(object, method = "pearson", adj.pval = FALSE,  
plot.proportions = FALSE)
```

**Arguments**

object	InTADSig object with signals and genes combined in TADS
method	Correlation method: "pearson" (default), "kendall", "spearman"
adj.pval	Perform p-value adjustment and include q-values in result
plot.proportions	Plot proportions of signals and genes in correlation

**Value**

A table with correlation values for signal-gene pairs including correlation p-value, euclidian distance and rank.

**Examples**

```
## perform analysis on test data  
inTadSig <- newSigInTAD(enhSel, enhSelGR, rpkmCountsSel, txsSel)  
inTadSig <- filterGeneExpr(inTadSig, geneType = "protein_coding")  
inTadSig <- combineInTAD(inTadSig, tadGR)  
corData <- findCorrelation(inTadSig, method="pearson")
```

---

fnSE	<i>Preparation for correlation analysis for a signal</i>
------	--

---

**Description**

This function collects all genes for signal genomic region inside of Topologically Associated Domains (TADs)

**Usage**

```
fnSE(id, sigList, tadGR, tss, pickMaxOvlp, nearestTad)
```

**Arguments**

id	Id of signal from the list
sigList	List of signal GRs and their names
tadGR	TAD genomic regions
tss	Gene transcription start sites
pickMaxOvlp	Use TAD with max overlap
nearestTad	The table listing TADs nearest to each TSS #'

**Details**

The signal is checked if it is lying inside of TAD. Then all genes in this TAD are collected.

**Value**

Data.frame containing genes connected to signal

---

geneCoords	<i>Gene coords GRanges</i>
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---

**Description**

This function returns the gene GRanges

**Usage**

```
geneCoords(object)

## S4 method for signature 'InTADSig'
geneCoords(object)
```

**Arguments**

object	InTADSig object with signals and genes
--------	--

**Value**

Gene GRanges

**Examples**

```
inTadSig <- newSigInTAD(enhSel, enhSelGR, rpkmCountsSel, txsSel)
head(geneCoords(inTadSig))
```



---

get.enr.bg.normfit      *Function to estimate gene expression*

---

### Description

This function uses mclust package to analyze gene expression distribution

### Usage

```
get.enr.bg.normfit(x)
```

### Arguments

x                      Full gene expression vector

### Details

The function adjust filtering cut value based on mclust library to exclude low expressed genes. It is a part of filtering procedure.

### Value

Distribution properties: mean and std

---

InTADSig                      *The InTADSig Class*

---

### Description

The InTADSig object stores signals and gene expression data for the samples.

### Details

It uses MultiAssayExperiment object to store information. Key slots to access are listed below.

### Slots

sigMAE: "MultiAssayExperiment", MultiAssayExperiment object containg signals and gene counts

signalConnections: "list", The list of signals representing gene data frames in the same TAD

loopsDf: "data.frame", The data.frame containing details of provided input loops

loopConnections: "list", The list of connections between signals and genes via loops

ncore: "numeric", Number of cores to use for parallel computing #'

---

loadSigInTAD	<i>Load InTADSig object from text files</i>
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---

### Description

The function loads the data tables to create an object that contains the signals and gene expression data.frames along with their genomic coordinates for further processing.

### Usage

```
loadSigInTAD(signalsFile, countsFile, gtffFile, annFile = "",  
             performLog = TRUE, logExprsOffset = 1, ncores = 1)
```

### Arguments

signalsFile	Tab-separated data table containing signals and their coordinates as row.names
countsFile	Tab-separated counts table
gtffFile	GTF file containing all gene coordinates
annFile	Tab-delimited phenotype annotation of samples
performLog	Perform log <sub>2</sub> conversion of expression values. Default: TRUE.
logExprsOffset	Offset x for log <sub>2</sub> gene expression i.e. log <sub>2</sub> (value + x). Default: 1
ncores	Number of cores to use for parallel computing

### Details

The function loads data from input files and creates object that stores matrices of signals and gene expression values along with coordinates. The samples order and names of columns should match in both tables. It is expected that gene ids are applied in the validation of counts table.

### Value

Novel InTADSig object

### Examples

```
# create sigInTAD object  
inTadSig <- newSigInTAD(enhSel, enhSelGR, rpkmCountsSel, txsSel)
```

---

loopsDfSel	<i>Data frame containing coordinates of loops</i>
------------	---

---

**Description**

The table contains genomic coordinates of chromatin loops in 6-column format derived from IMR90 cell line (focus : chr15)

**Usage**

```
loopsDfSel
```

**Format**

a data.frame object

**Value**

NULL, but makes available the dataset

---

mbAnnData	<i>Data frame containing information about samples</i>
-----------	--

---

**Description**

The table includes additional information about MB tumour samples (subgroup, gender, age, histology and M.Stage)

**Usage**

```
mbAnnData
```

**Format**

a data.frame object

**Value**

NULL, but makes available the dataset

---

newSigInTAD                      *Create InTADSig object*

---

### Description

The function generates an object that contains the signals and gene expression data.frames along with their genomic coordinates for further processing.

### Usage

```
newSigInTAD(signalData = NULL, signalRegions = NULL, countsData = NULL,  
            geneRegions = NULL, sampleInfo = NULL, performLog = TRUE,  
            logExprsOffset = 1, ncores = 1)
```

### Arguments

signalData	data frame containing signals
signalRegions	genomic regions of the signals
countsData	data matrix containing count expression values
geneRegions	gene coordinates
sampleInfo	data frame containing additional sample info
performLog	Perform log <sub>2</sub> conversion of expression values. Default: TRUE.
logExprsOffset	Offset x for log <sub>2</sub> gene expression i.e. log <sub>2</sub> (value + x). Default: 1
ncores	Number of cores to use for parallel computing

### Details

InTADSig object stores matrices of signals and gene expression values along with coordinates. The order of samples and names of columns should match in both datasets. For gene coordinates GRanges "gene\_id" and "gene\_name" are required in metadata. These are typical markers of genes in GTF annotation format.

### Value

Novel InTADSig object

### Examples

```
## create sigInTAD object  
inTadSig <- newSigInTAD(enhSel, enhSelGR, rpkmCountsSel, txsSel)
```

---

plotCorAcrossRef      *Function to plot correlation across genome*

---

### Description

This function creates a plot of correlation strength in target genomic region from the result table. The X-coordinates represent signals, Y-coords represent genes, while each dot represents  $-\log_{10}(P\text{-value})$  from correlation test. Additionally all TAD boundaries can be visualized.

### Usage

```
plotCorAcrossRef(obj, corRes, targetRegion, showCorVals = FALSE,
  symmetric = FALSE, tads = NULL)
```

### Arguments

obj	InTADSig object with signals and genes combined in TADS
corRes	Correlation result table created by function findCorrelation()
targetRegion	Target genomic region visualise.
showCorVals	Use this option to visualize positive correlation values instead of correlation strength
symmetric	Activate mirror symmetry for gene-signal connections
tads	TAD regions to visualize. By default only TADs present in correlation result table are applied (NULL value).

### Value

A ggplot object for visualization or customization.

### Examples

```
inTadSig <- newSigInTAD(enhSel, enhSelGR, rpkmCountsSel, txsSel)
inTadSig <- combineInTAD(inTadSig, tadGR)
corData <- findCorrelation(inTadSig, method="pearson")
plotCorAcrossRef(inTadSig, corData, GRanges("chr15:25000000-28000000"))
```

---

plotCorrelation      *Function to plot correlation*

---

### Description

This function creates a plot of selected pair signal-gene

### Usage

```
plotCorrelation(obj, sId, geneName, xLabel = "Gene expression",
  yLabel = "Signal enrichment", colByPhenotype = "",
  corMethod = "pearson")
```

**Arguments**

obj	InTADSig object with signals and genes combined in TADS
sId	Signal id based on genomic coordinates i.e. "chr:start-end"
geneName	Gene name to select. Based on "gene_name" attribute.
xLabel	The label to mark signal X-axis. Default: "Gene expression"
yLabel	The label to mark signal Y-axis. Default: "Signal enrichment"
colByPhenotype	The pheno data column i.e. tumour type that can be used for colour
corMethod	Correlation method. Default: Pearson

**Value**

A ggplot object for visualization or customization.

**Examples**

```
inTadSig <- newSigInTAD(enhSel, enhSelGR, rpkmCountsSel, txsSel)
inTadSig <- combineInTAD(inTadSig, tadGR)
plotCorrelation(inTadSig, "chr15:26372163-26398073", "GABRA5")
```

---

rpkmCountsSel

*Gene expression subset from medulloblastoma samples*

---

**Description**

This data.frame contains RPKM gene expression values from chr15 for subset from 25 medulloblastoma samples.

**Usage**

```
rpkmCountsSel
```

**Format**

a data.frame instance

**Value**

NULL, but makes available the dataframe

---

sigCoords	<i>Signal coords GRanges</i>
-----------	------------------------------

---

**Description**

This function returns the signal GRanges

**Usage**

```
sigCoords(object)

## S4 method for signature 'InTADSig'
sigCoords(object)
```

**Arguments**

object            InTADSig object with signals and genes

**Value**

Signal GRanges

**Examples**

```
inTadSig <- newSigInTAD(enhSel, enhSelGR, rpkmCountsSel, txsSel)
head(sigCoords(inTadSig))
```

---

signals	<i>Signal values table</i>
---------	----------------------------

---

**Description**

This function returns the signal values table

**Usage**

```
signals(object)

## S4 method for signature 'InTADSig'
signals(object)
```

**Arguments**

object            InTADSig object with signals and genes

**Value**

Signals table

**Examples**

```
inTadSig <- newSigInTAD(enhSel, enhSelGR, rpkmCountsSel, txsSel)
head(signals(inTadSig))
```

---

tadGR	<i>Genomic coordinates of topologically associated domains</i>
-------	--

---

**Description**

This GRanges object contains the coordinates of TADs revealed from IMR90 cell line (extracted from 0-indexed .bed file)

**Usage**

```
tadGR
```

**Format**

a GRanges object

**Value**

NULL, but makes available the dataset

---

txsSel	<i>Genomic coordinates of genes subset</i>
--------	--

---

**Description**

This GRanges object contains the coordinates of genes subset from chr15

**Usage**

```
txsSel
```

**Format**

a GRanges object

**Value**

NULL, but makes available the dataset



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