

# Package ‘CHRONOS’

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**Title** CHRONOS: A time-varying method for microRNA-mediated sub-pathway enrichment analysis

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**Description** A package used for efficient unraveling of the inherent dynamic properties of pathways. MicroRNA-mediated subpathway topologies are extracted and evaluated by exploiting the temporal transition and the fold change activity of the linked genes/microRNAs.

**Depends** R (>= 3.5)

**SystemRequirements** Java version >= 1.7, Pandoc

**License** GPL-2

**NeedsCompilation** no

**LazyLoad** yes

**Imports** XML, RCurl, RBGL, parallel, foreach, doParallel, openxlsx, igraph, circlize, graph, stats, utils, grDevices, graphics, methods, biomaRt, rJava

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**VignetteBuilder** knitr

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CHRONOSrun	<i>Default run of CHRONOS</i>
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---

### Description

Default run of CHRONOS

### Usage

```
CHRONOSrun(mRNAexp, mRNAlabel, miRNAexp, pathType, subType, measures,
           thresholds, org, export, verbose, miRNAinteractions)
```

### Arguments

mRNAexp	mRNA expressions filename located in CHRONOS/extdata/Input
mRNAlabel	mRNA nomenclature (for supported types see <a href="#">convertNomenclature</a> )
miRNAexp	miRNA expressions filename located in CHRONOS/extdata/Input
pathType	Pathway type ('Metabolic', 'Non-Metabolic', 'All' or vector of pathway ids)
subType	Subpathway type ('Linear', 'Non-Linear', 'All')
measures	Include subpathway structural and functional aspects ('TRUE', 'FALSE')
thresholds	Subscore, mirscore and p-value thresholds c('pvalue'=pvalue, 'subscore'=subscore, 'mirscore'=mirscore)
org	KEGG organism identifier
export	Export file type ('.xlsx', '.txt')
verbose	Show informative messages (TRUE/FALSE).
miRNAinteractions	Edgelist of miRNA-mRNA interactions.

**Details**

- Imports gene and miRNA expressions from CHRONOS/extdata/Input/<mRNAexpFile>.txt and CHRONOS/extdata/Input/<miRNAexpFile>.txt
- Downloads all available pathways for the specified organism from KEGG.
- Creates pathway graphs from downloaded KGML files.
- Extracts linear subpathways from metabolic and non metabolic graphs.
- Extracts non linear subpathways from metabolic and non metabolic graphs.
- Downloads miRecords miRNA-mRNA interactions.
- Scores and evaluates (linear and non linear) subpathways to extract significant results.
- Organism identifier.
- Visualizes most the significant results ('.xlsx' or '.txt').
- Display informative messages (TRUE/FALSE).
- User-defined miRNA-mRNA interactions can be supplied in the form of an edgelist with two columns. If no such information is available, a missing or a NULL argument forces the use of default interactions by using [downloadMiRecords](#).

**Value**

.

**Examples**

```
# Default run

load(system.file('extdata', 'Examples//data.RData', package='CHRONOS'))

res <- CHRONOSrun( mRNAexp=mRNAexpr,
                  mRNAlabel='entrezgene',
                  miRNAexp=miRNAexpr,
                  pathType=c('04915', '04917', '04930', '05031'),
                  org='hsa',
                  subType='Linear',
                  thresholds=c('subScore'=0.4, 'mirScore'=0.4),
                  miRNAinteractions=miRNAinteractions)
```

---

 convertMiRNANomenclature

*Conform miRNA annotations to the ones currently used by miRecords.*

---

**Description**

Conform miRNA annotations to the ones currently used by miRecords.

**Usage**

```
convertMiRNANomenclature(org, miRNAs, update)
```

**Arguments**

org	KEGG organism identifier.
miRNAs	Vector of miRNAs identifiers.
update	Update annotation mapper with latest annotation changes.

**Details**

Determine which miRNAs are incompatible with miRecords annotations and retrieve the suitable ones from [www.mirbase.org](http://www.mirbase.org).

**Value**

.

**Examples**

```
data <- c('hsa-let-7g-5p', 'hsa-miR-154-5p', 'hsa-miR-376b-3p')
convertMiRNANomenclature(org='hsa', miRNAs=data)
```

---

convertNomenclature    *Convert genes identifier nomenclature.*

---

**Description**

Convert genes identifier nomenclature.

**Usage**

```
convertNomenclature(ids, org, from, to)
```

**Arguments**

ids	Vector of gene identifiers
org	KEGG organism identifier
from	Initial identifier type
to	A vector of final identifier types

**Details**

EntrezGene ID	'entrezgene'
Ensembl Gene ID	'ensembl_gene_id'
Ensemble Transcript ID	'ensembl_transcript_id'
Ensemble Protein ID	'ensembl_peptide_id'
HGNC ID	'hgnc_id'
HGNC Symbol	'hgnc_symbol'
HGNC Transcript name	'hgnc_transcript_name'
Refseq mRNA ID	'refseq_mrna'

Refseq Protein ID	'refseq_peptide'
UniProt/Swissprot Accession	'uniprot_swissprot_accession'
UniProt/Swissprot ID	'uniprot_swissprot'
UniGene ID	'unigene'
UniProt Genename ID	'uniprot_genename'

**Value**

Vector of converted gene identifiers

**Examples**

```
# Identifiers to be converted
ids <- c('5091', '5105')

# Convert to HGNC ID, Ensembl Gene ID and UniProt Genename ID
from <- 'entrezgene'
to <- c('hgnc_symbol', 'ensembl_gene_id', 'uniprot_genename')
## Not run: res <- convertNomenclature(ids=ids, org='hsa', from=from, to=to)
```

---

createPathwayGraphs     *Convert KEGG Pathways to Gene-Gene Network Graphs.*

---

**Description**

Convert KEGG Pathways to Gene-Gene Network Graphs.

**Usage**

```
createPathwayGraphs(org, pathways, edgeTypes, doubleEdges, choice, groupMode)
```

**Arguments**

org	KEGG organism identifier.
pathways	Vector of KEGG pathway identifiers.
edgeTypes	Vector of edge types mappings.
doubleEdges	Specify which edgeTypes should be considered bidirectional.
choice	Create metabolic graph either by using relations or reactions from KGML file ('reactions', 'relations')
groupMode	'expand' to consider each group member a node, or 'collapse' to consider all components' genes as a node

**Details**

KEGG pathways consist of nodes each one containing one or more genes. Thus, two kinds of adjacency matrices are created. The compact adjacency matrix retains the groupings and stores edge types between genes and genes, genes and groups of genes or between group of genes. The expanded adjacency matrix stores edge type information between individual genes.

**Value**

A list containing a list of compact adjacency matrices, a list of expanded adjacency matrices, and list detailing all nodes, edges and interaction types.

**References**

Li, C., Han, J., Yao, Q., Zou, C., Xu, Y., Zhang, C., ... & Li, X. (2013). Subpathway-GM: identification of metabolic subpathways via joint power of interesting genes and metabolites and their topologies within pathways. *Nucleic acids research*, 41(9), e101-e101.

**Examples**

```
# Download Insulin Signaling Pathway
pathways <- c('04915', '04917', '04930', '05031')
paths    <- downloadPathways(org='hsa', pathways=pathways)

# Create pathway graph
graphs   <- createPathwayGraphs(org='hsa', pathways=paths)
```

---

downloadKEGGPathwayList

*Retrieve all available pathways for an organism.*

---

**Description**

Retrieve all available pathways for an organism.

**Usage**

```
downloadKEGGPathwayList(org)
```

**Arguments**

org                    KEGG organism identifier.

**Details**

.

**Value**

Data frame of pathway ids and names.

**References**

- <http://www.genome.jp/kegg/pathway.html>

## Examples

```
# Load extracted linear subpathways from toy data
load(system.file('extdata', 'Examples//data.RData', package='CHRONOS'))

# Retrieve all available hsa pathways
## Not run: pathways <- downloadKEGGPathwayList(org='hsa')
```

---

downloadMiRecords      *Download miRNA-mRNA interactions for an organism.*

---

## Description

Download miRNA-mRNA interactions for an organism.

## Usage

```
downloadMiRecords(org, pn, update, databases)
```

## Arguments

org	KEGG organism identifier.
pn	Number of databases that verify miRNA-mRNA interactions.
update	Download preprocessed data (update=FALSE) or new data from miRecords (update=TRUE).
databases	Specify which miRNA-mRNA interaction databases will be used.

## Details

miRecords is a resource for animal miRNA-target interactions. The Predicted Targets component of miRecords is an integration of predicted miRNA targets produced by 11 established miRNA target prediction tools, namely DIANA-microT, MicroInspector, miRanda, MirTarget2, miTarget, NBmiRTar, PicTar, PITA, RNA22, RNAhybrid, and TargetScan/TargertScanS.

## Value

Downloaded data is stored in CHRONOS/extdata/Downloads/miRecords/<org>/miRNATargets.RData

## References

- <http://c1.accurascience.com/miRecords>

## Examples

```
# Load extracted linear subpathways from toy data
load(system.file('extdata', 'Examples//data.RData', package='CHRONOS'))

## Not run: downloadMiRecords(org='hsa', pn=5, update=FALSE, databases='All')
```

---

downloadPathways      *Download KEGG pathways in KGML format.*

---

### Description

Download KEGG pathways in KGML format.

### Usage

```
downloadPathways(org, pathways)
```

### Arguments

org	KEGG organism identifier
pathways	Download pathways for specified organism:
	'All'    All organism pathways
	'Metabolic'    Metabolic pathways
	'Non-Metabolic'    Non metabolic pathways
<vector of indexes>	Using indexes from <a href="#">downloadKEGGPathwayList</a>
<vector of names>	Using pathway identifiers (i.e. c('00010', '00020'))

### Details

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a database resource for understanding high-level functions and utilities of the biological , system such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies.

Files are downloaded in CHRONOS/extdata/Downloads/KEGG/<org> folder.  
 Downloading is skipped for existing files.

### Value

Downloaded data is stored in CHRONOS/extdata/Downloads/KEGG/<org>

### References

- <http://www.genome.jp/kegg/pathway.html>

### Examples

```
# View all available hsa pathways
## Not run: pathways <- downloadKEGGPathwayList(org='hsa')

# Download pathway KGML files
pathways <- c('04915', '04917', '04930', '05031')

## Not run: pathways <- downloadPathways(org='hsa', pathways=pathways)
```



---

`extractLinearSubpathways`*Linear subpathway extraction from pathway graphs*

---

**Description**

Linear subpathway extraction from pathway graphs

**Usage**

```
extractLinearSubpathways(graphs, pathways, a, b, filter, export, groupMode,  
    verbose)
```

**Arguments**

<code>graphs</code>	Pathway graphs as returned from <a href="#">createPathwayGraphs</a> .
<code>pathways</code>	The subset of pathways from whom subpathways are to be extracted. If missing, all pathway graphs are used.
<code>a</code>	Minimum subpathway length.
<code>b</code>	Maximum subpathway length.
<code>filter</code>	Filter the subpaths with user genes (TRUE).
<code>export</code>	Exports subpaths in CHRONOS/extdata/Output/Subpaths/Linear/<org> folder. Available formats are '.txt' and/or '.RData'.
<code>groupMode</code>	Expand paralogues ('expand') or collapse them to a single entry ('collapse').
<code>verbose</code>	Display informative messages (TRUE) Requires previous execution of <a href="#">importExpressions</a> .

**Details**

Subpath filtering supports the removal of subpaths that have at least one member not belonging to the set of user supplied genes. These genes are extracted from the user's mRNA expressions matrix. Thus, the execution of [importExpressions](#) is a prerequisite.

To extract linear subpathways from a pathway graph, all possible start and end nodes are considered. A start node has only outgoing edges while an end node only has incoming edges. For each such pair, all linear subpathways are found by traversing the corresponding graph. Since the initial pathway graph's nodes contain one or more genes, resulting subpathways consist of bins of one or more genes. These subpaths are expanded to subpathways with one gene per bin in order to obtain usable subpathways.

**Value**

Returns a list consisting of

- A matrix of linear subpathways (subpaths)
- A list of processed pathway graphs adjacency matrices (`adjMats`)
- A list of processed pathway genes and interactions between them (`lexicon`)

**Examples**

```
# Load pathway graphs from toy data
load(system.file('extdata', 'Examples//data.RData', package='CHRONOS'))

# Extract linear subpathways
linSubs <- extractLinearSubpathways(graphs=graphs)
```

---

```
extractNonLinearSubpathways
```

*Non linear subpathway extraction from pathway graphs*

---

**Description**

Non linear subpathway extraction from pathway graphs

**Usage**

```
extractNonLinearSubpathways(graphs, pathways, a, b, k, filter, groupMode,
                             export, verbose)
```

**Arguments**

graphs	Pathway graphs as returned from <a href="#">createPathwayGraphs</a> .
pathways	The subset of pathways from whom subpathways are to be extracted. If missing, all pathway graphs are used.
a	Minimum subpathway length.
b	Maximum subpathway length.
k	Clique size.
filter	Filter the subpaths with user genes (TRUE).
groupMode	Expand paralogues ('expand') or collapse them to a single entry ('collapse').
export	Exports subpaths in CHRONOS/extdata/Output/Subpaths/Non-Linear/ <org> folder. Available formats are '.txt' and/or '.RData'.
verbose	Display informative messages (TRUE) Requires previous execution of <a href="#">importExpressions</a> .

**Value**

Returns a list consisting of

- A matrix of linear subpathways (subpaths)
- A list of processed pathway graphs adjacency matrices(adjMats)
- A list of processed pathway genes and interactions between them (lexicon)

To extract non linear subpaths from a pathway graph, all interactions between nodes of belonging to k-cliques are found. The ones that correspond

To extract non linear subpaths from a pathway graph, all interactions between nodes of belonging to k-cliques are found. The ones that correspond to actual interactions between genes make up the non linear subpath.

**Examples**

```
# Load pathway graphs from toy data
load(system.file('extdata', 'Examples//data.RData', package='CHRONOS'))

# Extract linear subpathways
nliSubs <- extractNonLinearSubpathways(graphs=graphs)
```

---

getEdgeTypes	<i>Map various types of gene-gene interactions in KGML files to edge types in corresponding pathway graphs.</i>
--------------	---

---

**Description**

Map various types of gene-gene interactions in KGML files to edge types in corresponding pathway graphs.

**Usage**

```
getEdgeTypes(type)
```

**Arguments**

type                    A vector of interaction types.

**Details**

Edge types

activation 1 inhibition 2 apathetic 3 no interaction 4

Default interaction - edge type mapping

01	unknown	3	02	activation	1
03	inhibition	2	04	binding/association	3
05	expression	1	06	repression	2
07	phosphorylation	3	08	dephosphorylation	3
09	ubiquitination	3	10	dissociation	3
11	indirect effect	3	12	state change	3
13	compound	3	14	hidden compound	3
16	missing interaction	3	16	activation_phosphorylation	1
17	activation_dephosphorylation	1	18	activation_ubiquitination	1
19	activation_indirect effect	1	20	activation_binding/association	1
21	activation_inhibition	3	22	activation_methylation	1
23	inhibition_phosphorylation	2	24	inhibition_dephosphorylation	2
25	inhibition_ubiquitination	2	26	inhibition_indirect effect	2
27	inhibition_binding/association	2	28	inhibition_expression	2
29	inhibition_methylation	2	30	compound_expression	1
31	compound_activation	1	32	compound_inhibition	2

33	compound_activation_indirect effect	1
34	compound_activation_phosphorylation	1
35	phosphorylation_indirect effect	3
36	phosphorylation_binding/association	3
37	phosphorylation_dissociation	3
38	dephosphorylation_indirect effect	3
39	binding/association_missing interaction	3
40	binding/association_indirect effect	3
41	expression_indirect effect	1
42	repression_indirect effect	2
43	ubiquitination_inhibition	2
44	dissociation_missing interaction	3
45	indirect effect_phosphorylation	3
46	activation_phosphorylation_binding/association	1
47	activation_phosphorylation_indirect effect	1

### Value

If an interaction type has been supplied, the corresponding edge types are returned. If not, the complete mapping is returned.

### Examples

```
# Example 1

# Retrieve edge types for phosphorylation and dephosphorylation.
getEdgeTypes(c(7,8))

# Example 2

# Returns a data frame containing the interaction - edge type mapper.
types <- getEdgeTypes()

# Set phosphorylation to inhibition.
types[8,2] <- 2
```

---

```
importExpressions      Import gene and miRNA expressions from
```

---

### Description

Import gene and miRNA expressions from

### Usage

```
importExpressions(data, type, sep, org, mRNAomenclature)
```

**Arguments**

data	Expressions data filename or matrix.
type	Expressions data type. (or mRNA expressions, type=<nomenType>. Available gene expression nomenclature can be found in <a href="#">convertNomenclature</a> . For miRNA expressions, type='miRNA').
sep	File delimiter.
org	KEGG organism identifier
mRNAomenclature	Nomenclature of user's mRNA expressions

**Details**

- Import gene expressions data from CHRONOS/extdata/Input/<userFile>.txt or a supplied matrix.
- Import miRNA expressions data from CHRONOS/extdata/Input/<userFile>.txt or a supplied matrix.

**Value**

.

**Examples**

```
# Example

load(system.file('extdata', 'Examples//data.RData', package='CHRONOS'))

importExpressions(data=mRNAexpr, type='mRNA',
                 mRNAomenclature='entrezgene', sep='\t', org='hsa')
importExpressions(data=miRNAexpr, type='miRNA', sep='\t', org='hsa')
```

---

pathwayMeasures      *Pathway structural and functional aspects*

---

**Description**

Pathway structural and functional aspects

**Usage**

```
pathwayMeasures(graphs)
```

**Arguments**

graphs      Pathway graphs as returned from [createPathwayGraphs](#).

**Details**

Structural and functional aspects of a pathway are calculated in respect to all organism pathways.

**Value**

Matrix with pathness, betweenness centrality and degree values for each gene in the pathway graphs at it's columns.

**Examples**

```
# Load pathway graphs from toy data
load(system.file('extdata', 'Examples//data.RData', package='CHRONOS'))

# Calculate pathway structural and functional aspects
measures <- pathwayMeasures(graphs)
```

---

scoreSubpathways	<i>Evaluate subpathways using an interacting scoring scheme (IS) for each time point.</i>
------------------	---

---

**Description**

Evaluate subpathways using an interacting scoring scheme (IS) for each time point.

**Usage**

```
scoreSubpathways(subpathways, filters, measures, parameters, miRNAinteractions)
```

**Arguments**

subpathways	Subpaths as returned from <a href="#">extractLinearSubpathways</a> and <a href="#">extractNonLinearSubpathways</a> .								
filters	Named vector of filters used for subpathway evaluation. Values denote corresponding thresholds.								
	<table> <tr> <td>pvalue</td> <td>Statistical evaluation</td> </tr> <tr> <td>measures</td> <td>Structural and functional evaluation</td> </tr> <tr> <td>subScore</td> <td>mRNA-mRNA interaction scoring</td> </tr> <tr> <td>mirScore</td> <td>miRNA-mRNA interaction scoring</td> </tr> </table>	pvalue	Statistical evaluation	measures	Structural and functional evaluation	subScore	mRNA-mRNA interaction scoring	mirScore	miRNA-mRNA interaction scoring
pvalue	Statistical evaluation								
measures	Structural and functional evaluation								
subScore	mRNA-mRNA interaction scoring								
mirScore	miRNA-mRNA interaction scoring								
measures	Subpathway structural and functional aspects as returned from <a href="#">pathwayMeasures</a> .								
parameters	C,K,T parameters of scoring scheme.								
miRNAinteractions	An edgelist of miRNA-mRNA interactions used to override downloaded interactions from miRecords.								

**Details**

...

**Value**

subpathways	High ranking subpathways
subScores	miRNA-subpathway scores
mRNAScores	mRNA-mRNA scores for each subpathway and for each time point
miRNAsOverSubpathway	High ranking miRNAs hitting each subpathway
pValues	P-value of each subpathway
filters	Filters used for the evaluation

## References

Jethava, V., Bhattacharyya, C., Dubhashi, D., & Vemuri, G. N. (2011). Netgem: Network embedded temporal generative model for gene expression data. *BMC bioinformatics*, 12(1), 327.

Kim, Y. et al. (2011). Principal network analysis: identification of subnetworks representing major dynamics using gene expression data. *Bioinformatics*, 27(3), 391-398

## Examples

```
# Load extracted subpathways from toy data
load(system.file('extdata', 'Examples//data.RData', package='CHRONOS'))

# Import mRNA expressions
mRNAexpr <- importExpressions(data=mRNAexpr, type='mRNA', org='hsa')

# Score extracted linear subpathways
filters <- c('subScore'=0.4)
linSubsScored <- scoreSubpathways(subpathways=linSubs, filters=filters)
```

---

subpathwayKEGGmap      *Create links to KEGG pathway map with highlighted subpathways.*

---

## Description

Create links to KEGG pathway map with highlighted subpathways.

## Usage

```
subpathwayKEGGmap(subpathways, type, openInBrowser)
```

## Arguments

subpathways	Subpathways as returned by <a href="#">extractLinearSubpathways</a> or <a href="#">extractNonLinearSubpathways</a>
type	Subpathway type (Linear, Non-Linear)
openInBrowser	Open link in default browser.

## Value

Vector of links of KEGG pathway maps.

**Examples**

```
# Load extracted linear subpathways from toy data
load(system.file('extdata', 'Examples//data.RData', package='CHRONOS'))

# Opening selected subpathways in default browser
subs <- linSubs$subpaths[1:3, ]

subpathwayKEGGmap(subpathways=subs, type='Linear', openInBrowser=FALSE)
```

---

subpathwayMiRNAs      *Create a circular plot of a subpathway and the miRNAs that target it.*

---

**Description**

Create a circular plot of a subpathway and the miRNAs that target it.

**Usage**

```
subpathwayMiRNAs(summary, subIdx, timePoints)
```

**Arguments**

summary	Output from <a href="#">scoreSubpathways</a>
subIdx	Subpathway index
timePoints	Time points to include in visualization, default to all.

**Value**

.

**Examples**

```
# Load scored subpathways from toy data
load(system.file('extdata', 'Examples//data.RData', package='CHRONOS'))
# Visualize one or more subpathways.
subpathwayMiRNAs(summary=linSubsScored, subIdx=2)
```

---

visualizeResults      *Visualize results in tabular form (txt, xls)*

---

**Description**

Visualize results in tabular form (txt, xls)

**Usage**

```
visualizeResults(summary, export, expand, colors, from, to)
```



**Arguments**

summary	Evaluation results as returned from <a href="#">scoreSubpathways</a>
export	'xlsx' exports a xlsx file and '.txt' a .txt file.
expand	TRUE if each subpathway member and miRNA belongs to a single cell, FALSE if all subpathway members belong to one cell and miRNAs to another cell.
colors	The color scheme used in subScores heatmap.
from	Primary annotation <a href="#">convertNomenclature</a> . Defaults to EntrezGene ID.
to	Secondary annotation <a href="#">convertNomenclature</a>

**Value**

A txt or a xlsx file in CHRONOS/extdata/Output/Scores/Linear/<org>  
or CHRONOS/extdata/Output/Scores/Non-Linear/<org>

**Examples**

```
# Load scored subpathways from toy data
load(system.file('extdata', 'Examples//data.RData', package='CHRONOS'))

visualizeResults(linSubsScored, export='txt')
```

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