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

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Table of Contents

Package Setup

CHRONOS (time-vaRying enriCHment integrOmics Subpathway aNalysis tOol) is an R package built to extract regulatory subpathways along with their miRNA regulators at each time point based on KEGG pathway maps and user-defined time series mRNA and microRNA (if available) expression profiles for microarray experiments. It is flexible by allowing the user to intervene and adapt all discrete phases to the needs of the study under investigation. CHRONOS can assist significantly in complex disease analysis by enabling the experimentalists to shift from the dynamic to the more realistic time-varying view of the involved perturbed mechanisms.

Before loading the package, please specify a user-accessible home directory. The default directories for each architecture are as follows:

```
if (.Platform$OS.type == 'unix')
    { options('CHRONOS_CACHE'=file.path(path.expand("~"), '.CHRONOS') ) }
if (.Platform$OS.type == 'windows')
    { options('CHRONOS_CACHE'=file.path(gsub("\\\\", "/",
                        Sys.getenv("USERPROFILE")), "AppData/.CHRONOS")) }
```

User Input

CHRONOS requires mRNA and microRNA (if available) time-series expression data along with their labels. The expression data needs to be formatted in matrices with dimensions (N, E), where N is the number of mRNAs/ microRNAs and E the time points of data (i.e. X(i,j) is the expression value of mRNA/miRNA i at time j). In case microRNA expression data are not available, CHRONOS can be run without processing and exporting miRNAs in the final subpathways. CHRONOS operates more effectively if data are normalized and log2-fold change differences relative to an initial condition (control state) are computed. Multiple biological replicates should be summarized so that one sample per time point is provided as input. An indicative example is:

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

```
##             [,1]        [,2]         [,3]          [,4]         [,5]
## 18 -0.0301981 -0.13214159  0.16098142  0.105607748  0.14038253
## 32  0.1002393  0.11929417 -0.15358686  0.512760639 -0.07647562
## 35  0.1678181  0.17534780  0.33999395  0.454677105  0.05409575
## 37  1.3563976  0.92800903 -0.65185547 -0.970592022 -0.14037323
## 39 -0.1676469 -0.11763173 -0.10790169 -0.002826691 -0.01870847
## 60  0.0373764 -0.05872822  0.01590395  0.224429607 -0.02530050
```

Default Run

Next we present a default run of CHRONOS, which (i) imports mRNA from CHRONOS/extdata/Input/.txt and miRNA expressions from CHRONOS/extdata/Input/.txt, (ii) downloads (all availiable) pathways for a specified organism from KEGG, (iii) creates pathway graphs from downloaded KGML files, (iv) extracts linear subpathways from metabolic and non metabolic graphs, (v) downloads miRecords miRNA-mRNA interactions, (vi) scores extracted subpathways in order to extract significant results, (vii) visualizes the most the significant results.

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

SubId	Pathway	Subscores					miRNA Mediated Subpathway Members (HGNC symbol)					miRNAs			
		T1	T2	T3	T4	T5									
S1	(hsa04915) Estrogen signaling pathway	0.34	0.335	0.583	0.463	0.387	CALM1	NOS3	ATF6B						
S2	(hsa04917) Prolactin signaling pathway	0.352	0.37	0.431	0.561	0.356	SOCS4	STAT1	CCND2						
S3	(hsa04915) Estrogen signaling pathway	0.339	0.387	0.568	0.464	0.344	CALM1	NOS3	CREB5						
S4	(hsa05031) Amphetamine addiction	0.21	0.402	0.555	0.391	0.359	CALML3	CAMK2A	CREB5	FOS		hsa-miR-101(t2)	hsa-miR-221(t3)		
S5	(hsa05031) Amphetamine addiction	0.126	0.415	0.559	0.407	0.344	CALM1	CAMK2A	CREB5	FOS		hsa-miR-101(t2)	hsa-miR-221(t3)		
S6	(hsa04917) Prolactin signaling pathway	0.356	0.198	0.539	0.574	0.155	SOCS3	STAT1	CCND2			hsa-miR-203(t2,t3,t4)	hsa-miR-30b(t3)		
S7	(hsa04930) Type II diabetes mellitus	0.442	0.427	0.306	0.26	0.379	TNF	MAPK8	IRS2	PIK3R3	SLC2A4	hsa-miR-181a(t1,t3)	hsa-miR-181c(t1,t3,t4)	hsa-miR-181b(t2)	
S8	(hsa04915) Estrogen signaling pathway	0.196	0.178	0.576	0.426	0.416	CALML3	NOS3	ATF6B						
S9	(hsa04915) Estrogen signaling pathway	0.285	0.157	0.618	0.422	0.311	CALM1	NOS3	CREB1			hsa-miR-27a(t3)	hsa-miR-27b(t3)		
S10	(hsa04915) Estrogen signaling pathway	0.195	0.23	0.562	0.427	0.372	CALML3	NOS3	CREB5						
S11	(hsa04917) Prolactin signaling pathway	0.389	0.198	0.519	0.516	0.155	SOCS2	STAT1	CCND2						
S12	(hsa04930) Type II diabetes mellitus	0.438	0.437	0.289	0.23	0.35	TNF	MAPK8	IRS2	PIK3CD	SLC2A4				
S13	(hsa05031) Amphetamine addiction	0.164	0.272	0.626	0.336	0.313	CALML3	CAMK2A	CREB1	FOS		hsa-miR-27a(t3)	hsa-miR-27b(t3)	hsa-miR-101(t2)	hsa-miR-221(t3)
S14	(hsa04917) Prolactin signaling pathway	0.394	0.396	0.161	0.283	0.457	SOCS4	STAT1	GCK						
S15	(hsa05031) Amphetamine addiction	0.081	0.285	0.631	0.353	0.297	CALM1	CAMK2A	CREB1	FOS		hsa-miR-27a(t3)	hsa-miR-27b(t3)	hsa-miR-101(t2)	hsa-miR-221(t3)
S16	(hsa04930) Type II diabetes mellitus	0.418	0.392	0.304	0.064	0.365	TNF	MAPK8	IRS2	PIK3CA	SLC2A4				
S17	(hsa04930) Type II diabetes mellitus	0.368	0.409	0.188	0.221	0.302	PRKCE	IRS2	PIK3CD	SLC2A4		hsa-miR-181a(t1)	hsa-miR-181c(t1,t3)		
S18	(hsa04915) Estrogen signaling pathway	0.14	0	0.612	0.385	0.339	CALML3	NOS3	CREB1			hsa-miR-27a(t3)	hsa-miR-27b(t3)		
S19	(hsa04917) Prolactin signaling pathway	0.431	0.224	0.25	0.239	0.255	SOCS2	STAT1	GCK						
S20	(hsa04930) Type II diabetes mellitus	0.259	0.426	0.118	0.262	0.232	SOCS3	IRS2	PIK3R3	SLC2A4		hsa-miR-203(t2,t3,t4)	hsa-miR-30b(t3)	hsa-miR-181a(t1,t3)	hsa-miR-181c(t1,t3,t4)	hsa-miR-181b(t2)
S21	(hsa04930) Type II diabetes mellitus	0.254	0.439	0.095	0.221	0.193	SOCS3	IRS2	PIK3CD	SLC2A4		hsa-miR-203(t2,t3,t4)	hsa-miR-30b(t3)	hsa-miR-181a(t1,t3)	hsa-miR-181c(t1,t3)

Figure 1: Results

Citation

The CHRONOS software package itself can be cited as: Vrahatis, A. G., Dimitrakopoulou, K., Balomenos, P., Tsakalidis, A. K., & Bezerianos, A. (2015). CHRONOS: A time-varying method for microRNA-mediated sub-pathway enrichment analysis. Bioinformatics (2016) 32 (6) : 884-892