

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

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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)
```

| 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 | SOC54 | 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 | CAMIC2A | 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 | CAMIC2A | 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 | SOC53 | 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 | (hsa04917) Prolactin 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 | SOC52 | 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 | CAMIC2A | CREB1 | FOS | hsa-miR-27a(t3) | hsa-miR-27b(t3) | hsa-miR-101(t2) | hsa-miR-221(t5) | | |
| S14 | (hsa04917) Prolactin signaling pathway | 0.394 | 0.396 | 0.161 | 0.283 | 0.457 | SOC54 | STAT1 | GCK | | | | | | | |
| S15 | (hsa05031) Amphetamine addiction | 0.081 | 0.285 | 0.631 | 0.353 | 0.297 | CALM1 | CAMIC2A | 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 | SOC52 | STAT1 | GCK | | | | | | | |
| S20 | (hsa04930) Type II diabetes mellitus | 0.259 | 0.426 | 0.118 | 0.262 | 0.232 | SOC53 | 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 | SOC53 | 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

```
##          [,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 available) 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='.txt')
```

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