The analysis of Time Series gene expression data

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Time-Series

- A series of values of variables taken in successive periods in time

- Time points
- Sampling intervals (constant/inconstant)

Gene expression is highly dynamic

- Biological processes are highly dynamic and are observed through the change of gene expressions
- To understand the molecular biological dynamics of specific biological process, gene expression must be observed at the most crucial time points
- A series of such gene expression snapshots is defined as Time-Series data

Power of Time Series Data Analysis

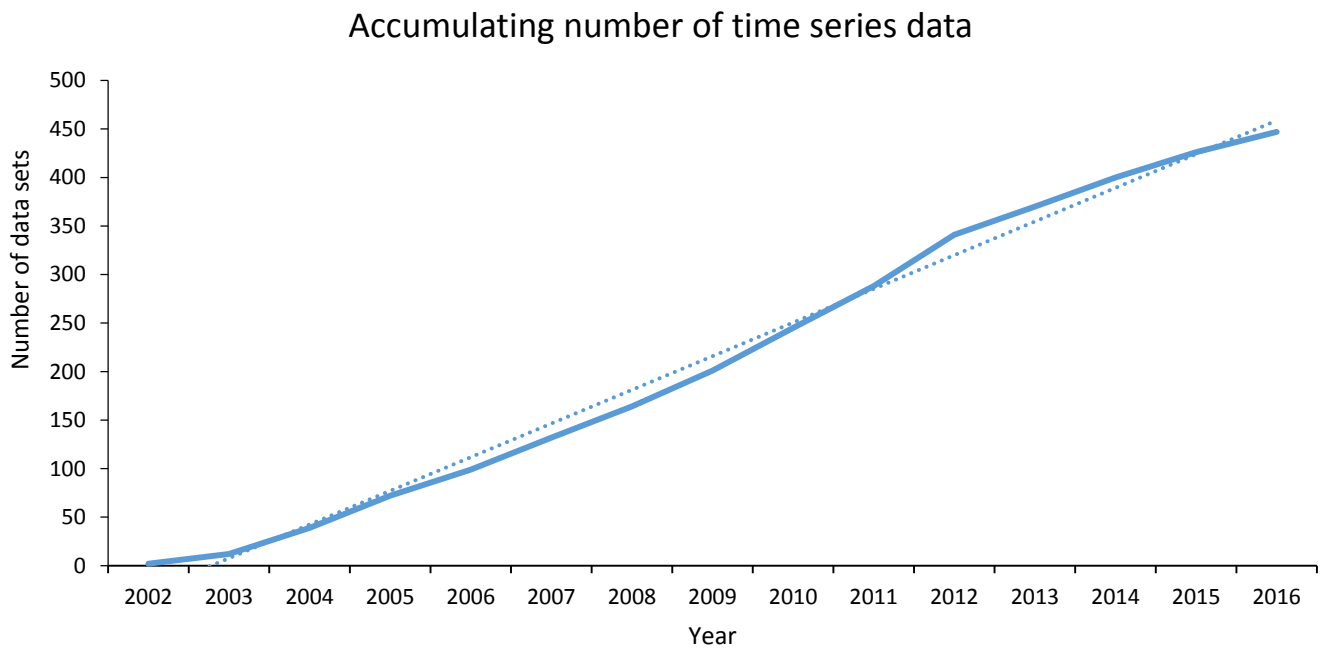
- Capture the molecular biological dynamics to understand the model of specific biological processes involved with transient expression change

- Transient expression change is observed in
  - developmental or cycling processes
  - perturbation-response experiments

- Such information is important for understanding
  - the sequence of events (activation of genes → causality)
  - detect temporal pattern of a response
  - the dynamic use of transcriptional networks

Exponentially increasing Time Series data

- Statistics of available time series in GEO
- Only recently, the number of time series NGS data are starting to increase



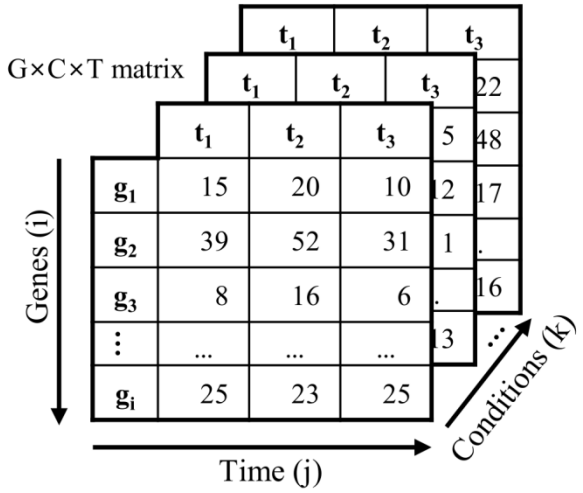
Biological Challenges

- Synchronization
- Duration and sampling rate
  - Developmental and cyclic systems
    - For cyclic processes, it should be uniform and cover multiple cycles
    - For developmental processes, there are two approaches
      - Morphological markers
      - Vary sampling rate during the life cycle according to the expected changes in gene expression (one-hour intervals during embryonic stages, multi-day intervals during adulthood in the D. melanogaster)
  - Perturbation-response experiments
    - Early time points are more important than later time points
- Sampling density
  - If interest is in identifying genes that take part in dynamics, more time points with fever replicates
  - If differentially expressed genes are important at certain time points, fewer time points with more replicates

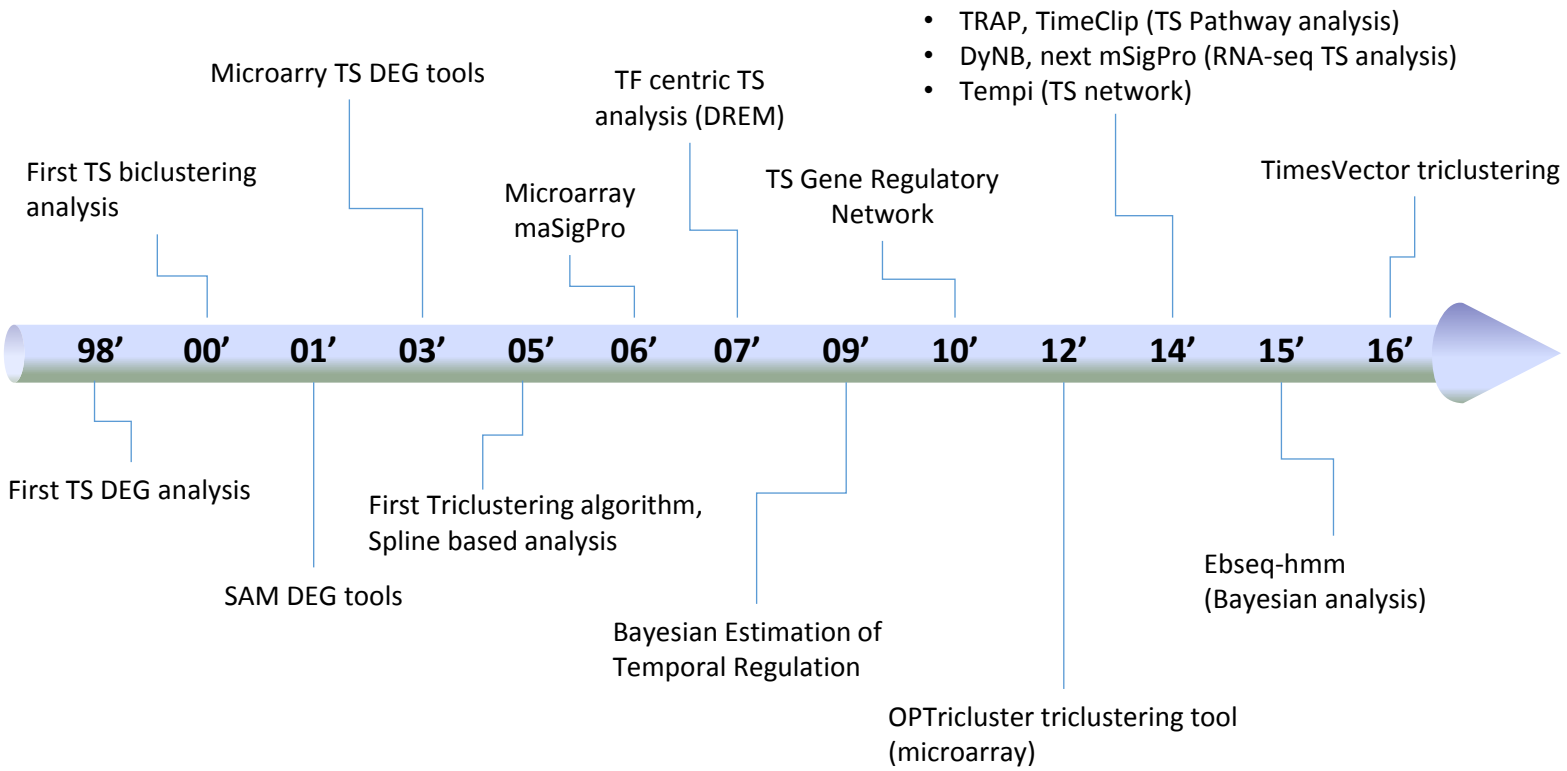
Computation Challenges

- A wide range of aspects for analysis (each being very difficult)
  - Single DEG detection
  - DEG clustering
  - Network
  - Pathway

- Data is diverse, large and complex
  - Microarray, NGS (normalization issues)
  - High dimensional (Gene-Time-Condition)



Timeline of Time-Series (TS) analysis milestones



Influence maximization in time bounded network identifies transcription factors regulating perturbed pathways

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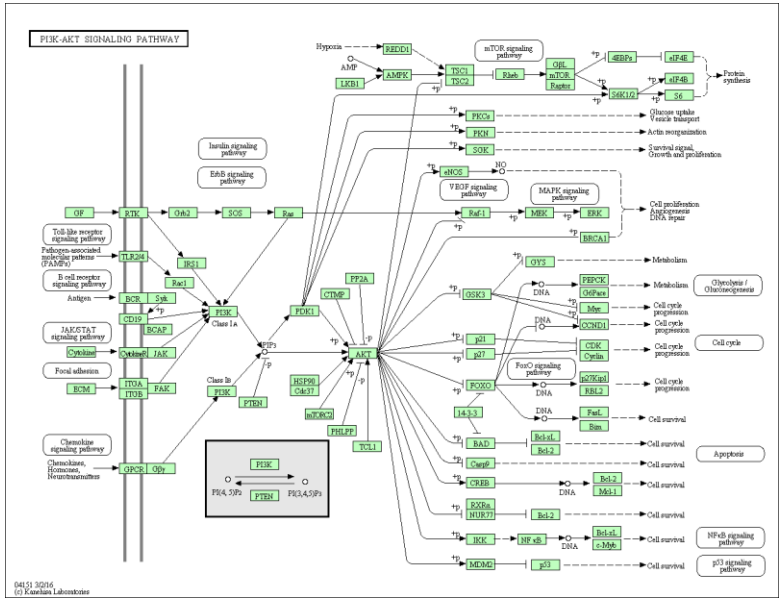
Background:
Biological Pathway

- An ordered series of molecular events that leads to a new molecular product, or a change in a cellular state

1.1 Carbohydrate metabolism
Glycolysis / Gluconeogenesis
Citrate cycle (TCA cycle)
Pentose phosphate pathway
Pentose and glucuronate interconversions
Fructose and mannose metabolism
Galactose metabolism
Ascorbate and aldarate metabolism
Starch and sucrose metabolism
Amino sugar and nucleotide sugar metabolism
Pyruvate metabolism
Glyoxylate and dicarboxylate metabolism
Propanoate metabolism
Butanoate metabolism
C5-Branched dibasic acid metabolism
Inositol phosphate metabolism
1.2 Energy metabolism
Oxidative phosphorylation
Photosynthesis
Photosynthesis - antenna proteins
Carbon fixation in photosynthetic organisms
Carbon fixation pathways in prokaryotes
Methane metabolism
Nitrogen metabolism
Sulfur metabolism
1.3 Lipid metabolism
Fatty acid biosynthesis
Fatty acid elongation
Fatty acid degradation
Synthesis and degradation of ketone bodies
Cutin, suberine and wax biosynthesis
Steroid biosynthesis
...

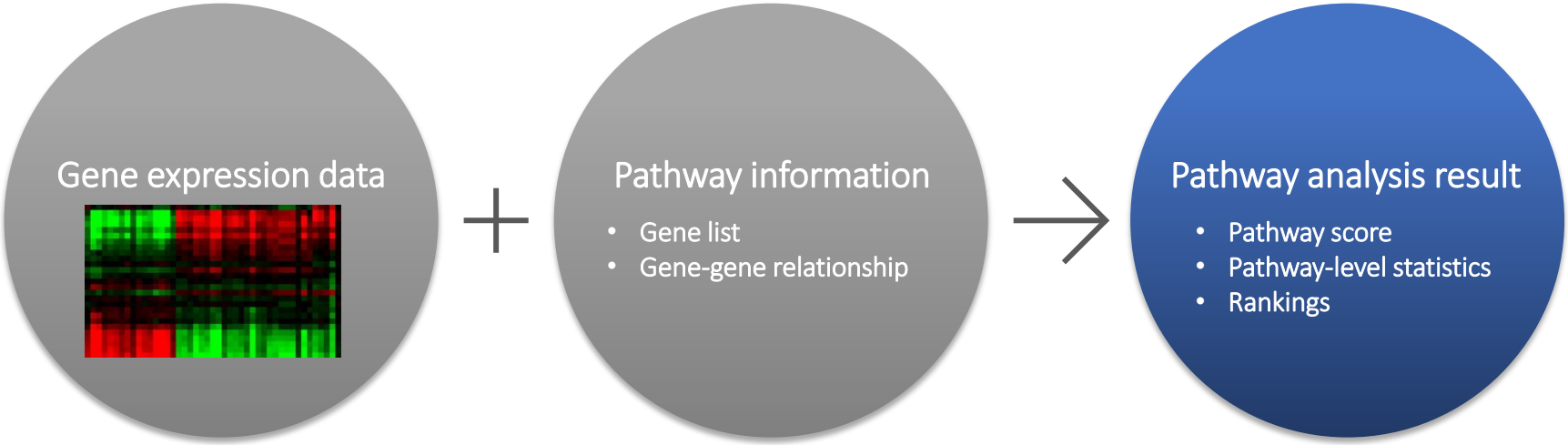
List of pathways (KEGG database)



PI3K/AKT signaling pathway (KEGG database)

Background:
Pathway analysis

- Pathway analysis identifies dysregulated (perturbed) biological pathways by stimuli or disease conditions

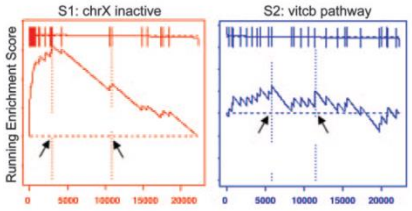


Background:
Timeline of Pathway analysis

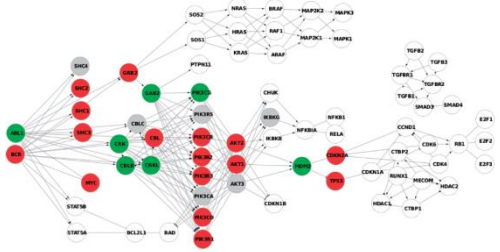
Gene-based Approach	Graph(Interaction)-based Approach	
ORA (2002)	Pathway-Express (2007)	DEGraph (2011)
GSEA (2005)	SPIA (2009)	Clipper (2013)
GSEA-SNP (2008)	NetGSA (2009)	DEAP (2013)
GSA-SNP (2010)		TEAK (2013)
		PATHiWAYs (2013)
	Pathway	Sub-pathway

No pathway tool for time-series

- Proportion of DEGs in a pathway
- Gene-level statistics (e.g. P-value) for individual genes



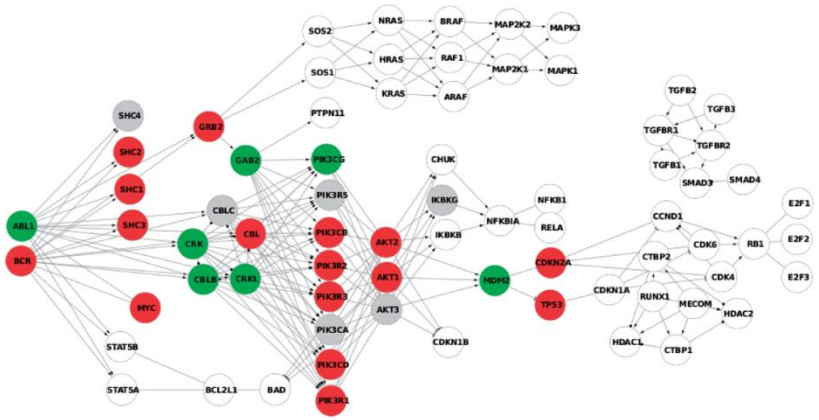
- Graph-based (node: gene / edge: interaction)
- Gene-gene relationship
- Pathway to sub-pathway



Motivation

- Q1. Sub-pathways from time-series transcriptome data ?

Transcriptome data



- Mapping DEGs in the pathway

Time-series Transcriptome data

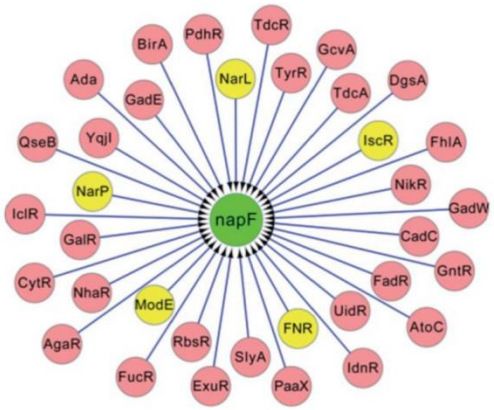
?

- DEGs from each time point or whole time points?
- How to detect expression propagation along time?

Motivation (cont.)

- Q2. Regulators of the sub-pathways from time-series transcriptome data ?

Transcriptome data



- Finding relationship between TF and target gene by their expression pattern

Time-series Transcriptome data

?

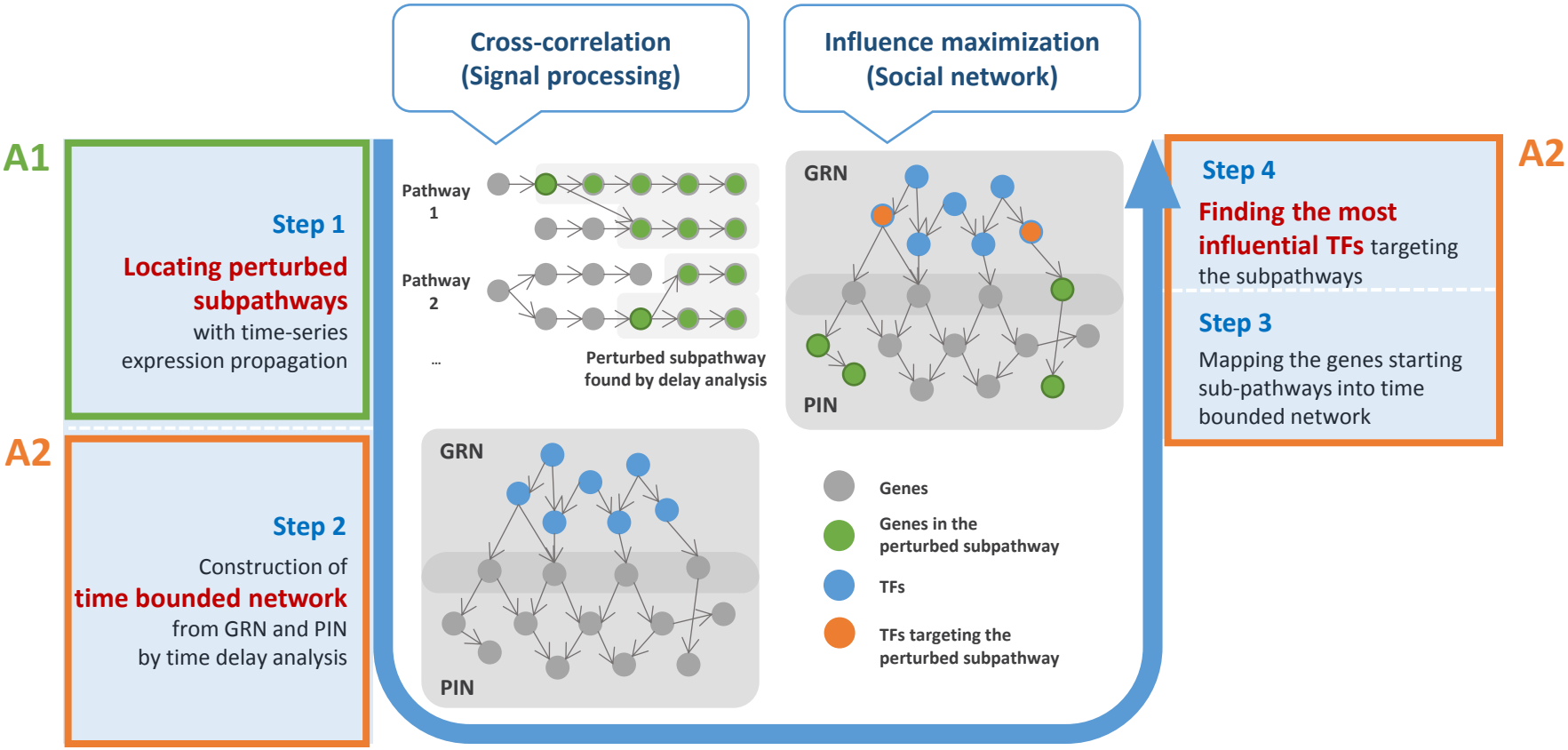
- What if there is a delay between TF-gene expression patterns?
- What if the number of pathway genes & candidate TFs are too large?

Two Main Ideas

- Q1. Sub-pathways from time-series transcriptome data ?
  - A1. **Cross-correlation** calculation between two differential expression vectors
- Q2. Regulators of the sub-pathways from time-series transcriptome data ?
  - A2. **Influence maximization** in the time bounded network

TimeTP (Time-series TF-Pathway map)

- Overview



Two Main Ideas

- Q1. Sub-pathways from time-series transcriptome data ?
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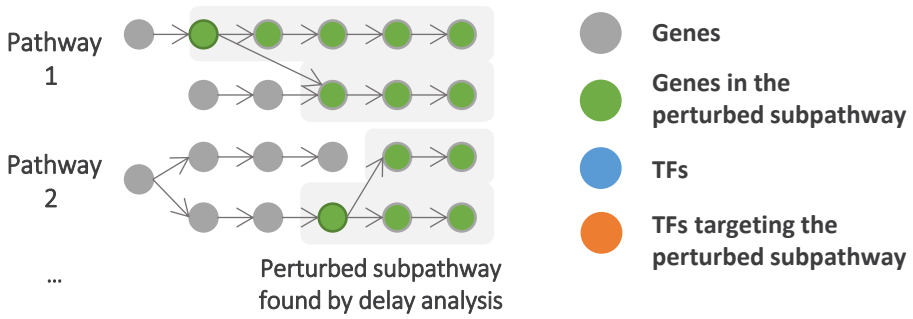
- Q2. Regulators of the sub-pathways from time-series transcriptome data ?
  - A2. **Influence maximization** in the time bounded network

Differential Expression Vectors

Step 1

Locating perturbed sub-pathways
with time-series expression propagation



- KEGG pathway database
- Pathway network is represented as a directed graph G=(N, E)
- TimeTP assigns a vector $\vec{v}$ for each node, representing the differential expression as 1 (overexpressed), -1 (underexpressed), or 0.

		T1	T2	T3	T4	T5
Gene expression value	Condition 1	30	23	40	101	90
	Condition 2	128	20	40	32	38
Differential expression vector		1	**0**	**0**	**-1**	**-1**

- Determined by Limma (microarray) or DEseq2 (RNA-seq) software

Locating perturbed sub-pathways

- For each pathway graph, TimeTP filters out invalid edges
- Validity of edges
  - Cross-correlation → **(1) direction of propagation**
    (2) the number of delayed time points for a gene pair
  - Direction of propagation should be same as the original graph
  - The number of delayed time points should be below the threshold

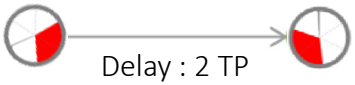
$$(\vec{v_1} \star \vec{v_2})(n) = \sum_{t=-\infty}^{\infty} \vec{v_1}(t)\vec{v_2}(t+n)$$

Cross-correlation between two genes

$$d(\vec{v_1}, \vec{v_2}) = \underset{n}{\operatorname{argmax}}(\vec{v_1} \star \vec{v_2})(n)$$

Delay between two genes

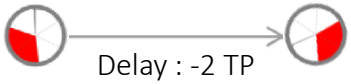
(a)



(0, 1, 1, 0, 0, 0) (0, 0, 0, 1, 1, 0)

Valid edge (0 < delay < threshold)

(b)



(0, 0, 0, 1, 1, 0) (0, 1, 1, 0, 0, 0)

Invalid edge (Delay < 0)

Two Main Ideas

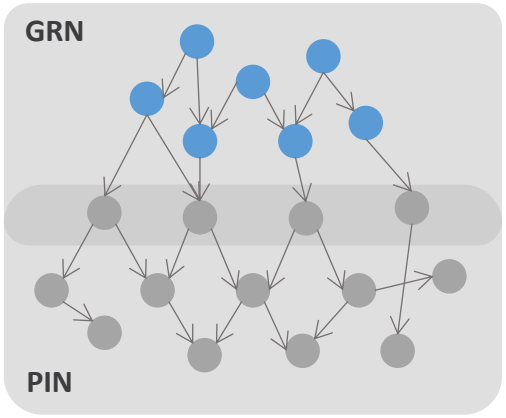
- Q1. Sub-pathways from time-series transcriptome data ?
  - A1. **Cross-correlation** calculation between two differential expression vectors

- Q2. Regulators of the sub-pathways from time-series transcriptome data ?
  - A2. **Influence maximization** in the time bounded network

Time-bounded network construction

Step 2

Construction of
time bounded network
from GRN and PIN
by time delay analysis



Genes
Genes in the perturbed subpathway
TFs
TFs targeting the perturbed subpathway

- Integration of GRN and PIN
  - **To search for upstream regulators of perturbed sub-pathways**, gene regulatory network (GRN) and protein-protein interaction network (PIN) are integrated.
  - HTRIdb (6 public databases and literature) and STRING database
  - Invalid edges are filtered by the cross-correlation

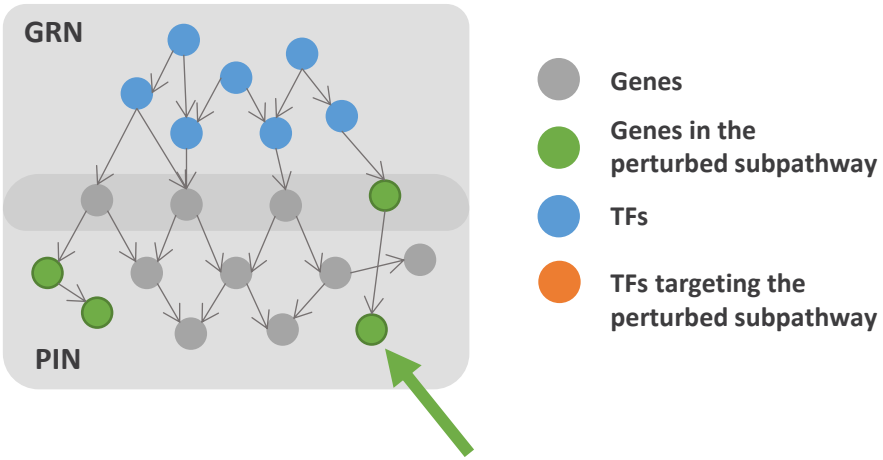
Labeled influence maximization for transcription factor detection

Step 4

Finding the most influential regulators targeting the subpathways

Step 3

Mapping the genes starting sub-pathways into time bounded network



- Mapping sub-pathway genes into the network
  - **Labeling the starting point of the perturbation** in the integrated network
  - To find the regulators that have the overall effect on multiple sub-pathways

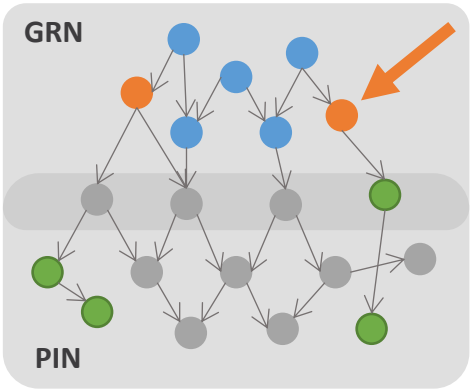
Labeled influence maximization for transcription factor detection (cont.)

Step 4

Finding the most influential regulators targeting the subpathways

Step 3

Mapping the genes starting sub-pathways into time bounded network



Genes

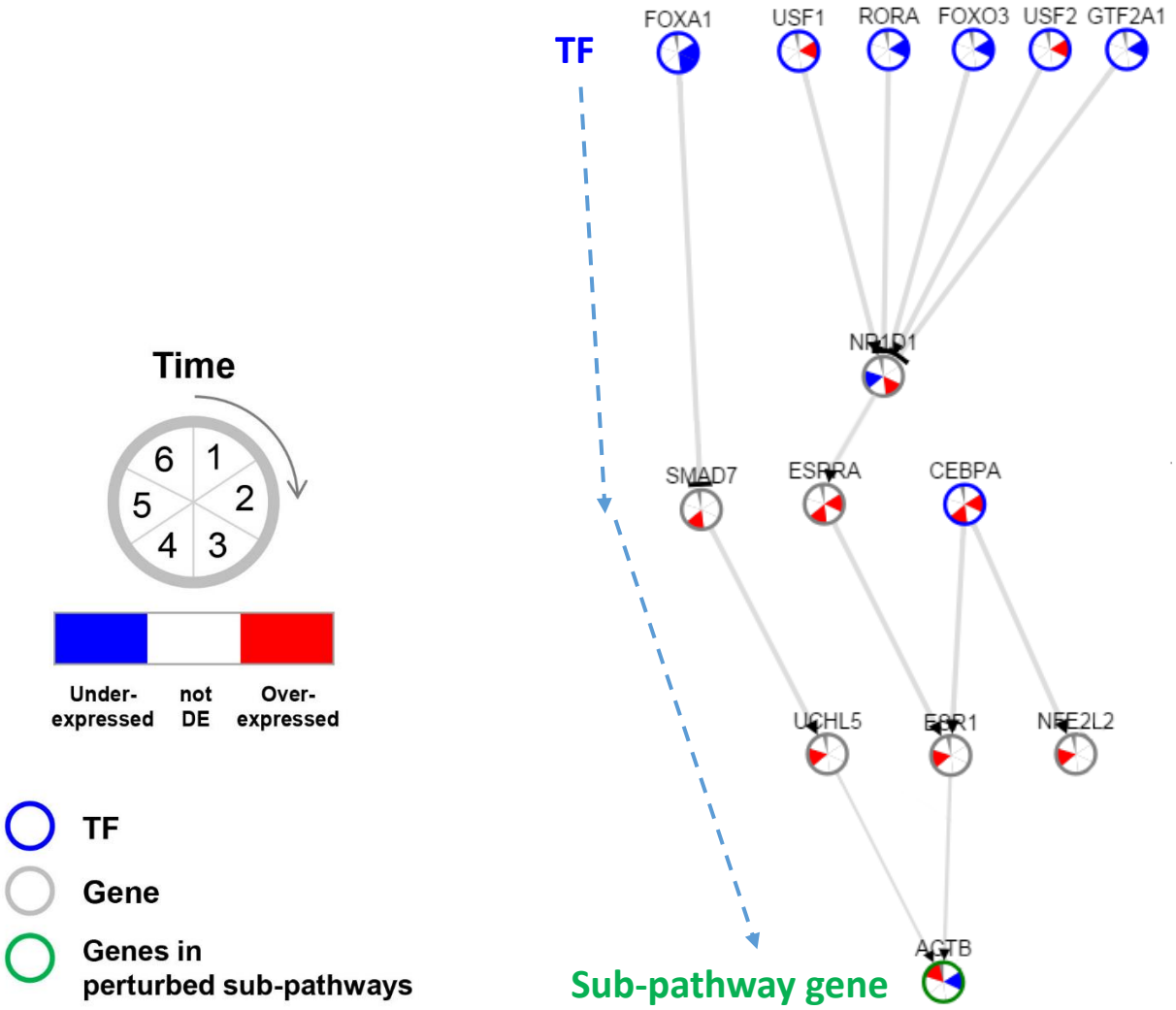
Genes in the perturbed subpathway

TFs

TFs targeting the perturbed subpathway

- Influence maximization
  - Used for social network to find a viral marketing targets that have the biggest influence to other customers
  - Labeled influence maximization (2011)
    - Finding the most influential node for specific (labeled) nodes
  - Given the starting points of the perturbed sub-pathways as labeled, **TimeTP finds the most influential TFs on sub-pathway genes**
  - **Scoring and ranking TFs by the amount of influence**

Visualization: TF-Pathway Map in Time Clock



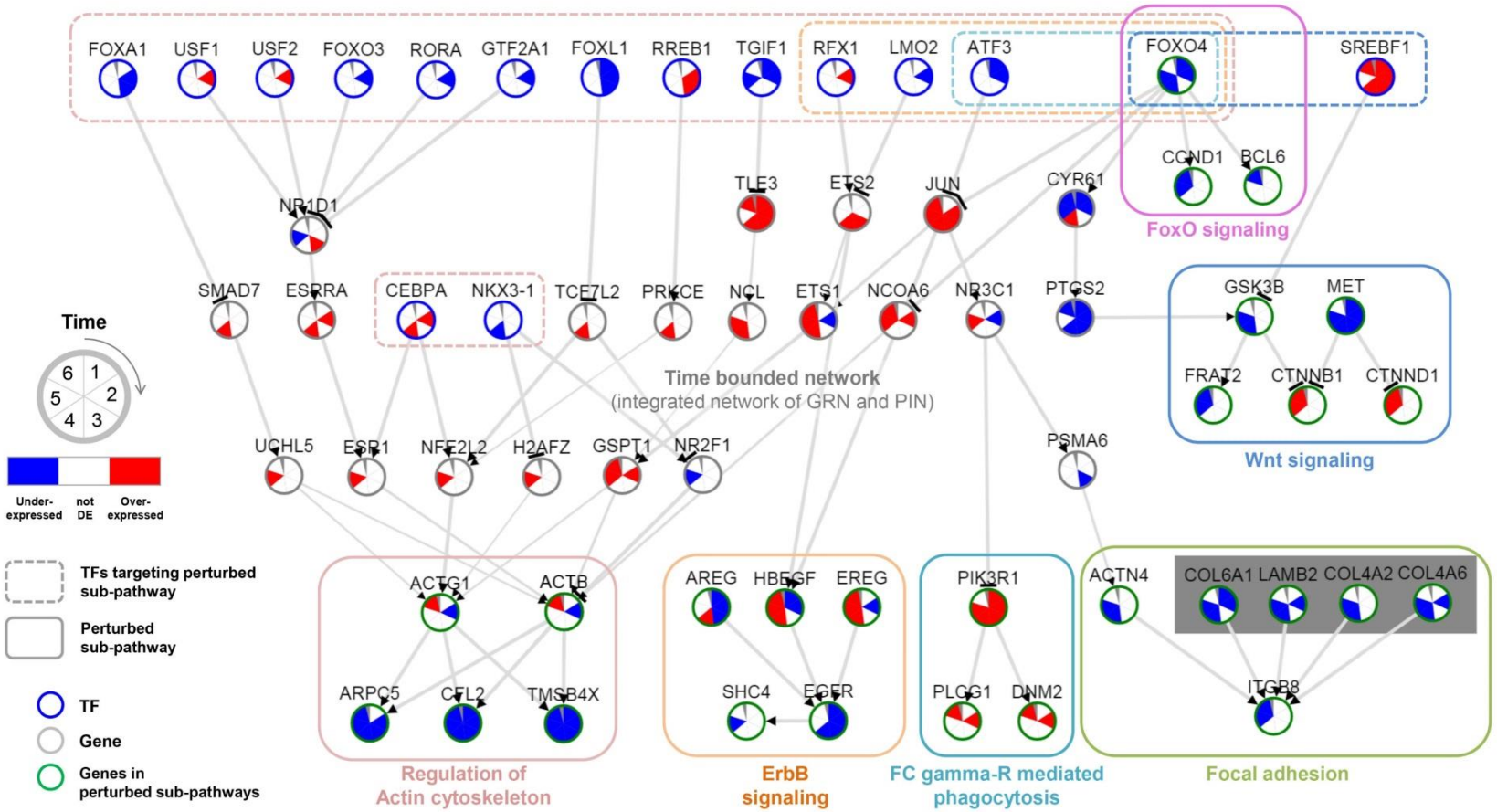
Test with Experimental Data

- MCF10A dataset
  - RNA-seq dataset of non-transformed human breast epithelial cells MCF10A (Kselev et al., 2015)
  - Stimulated with 10 ng/ml EGF(Epidermal growth factor)
    for 15, 40, 90, 180 and 300 min (6 time points including 0 min)
  - WT and PIK3CA knock-in samples to compare
  - Designed to trigger the long term activation of PIP3 signaling by the modification of PIK3CA and track its downstream effect

- EWS/FLI1 Knock-down dataset
  - Microarray dataset of a shRNA-induced EWS/FLI1 knockdown in the A673 Ewing's Sarcoma cell line (Bilke et al., 2013)
  - 6 time points including 0 min
  - Single time-series samples
  - EWS/FLI1: Ewing sarcoma oncoprotein
  - Designed to show a co-regulation of EWS/FLI1 and E2F3

TF-Pathway Map in Time Clock

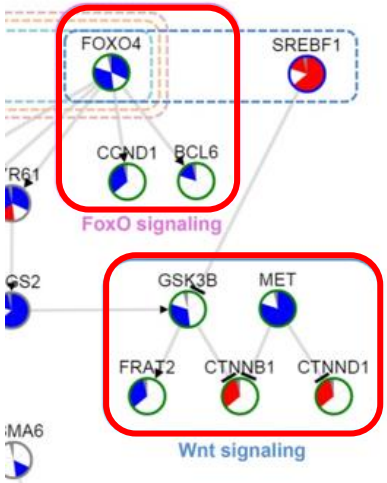
- MCF10A dataset

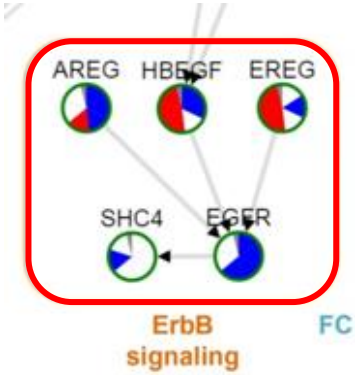


TF-Pathway Map in Time Clock

- **Findings**
  - **Perturbation in PI3K-Akt signaling**, the main objective of the biological experiment
  - Consequent changes in the downstream pathways of PI3K-Akt
  - Major findings supported by the previous studies:

1. **FOXO4 (Known targets of Akt)**
2. **FoxO and Wnt signaling pathway** (known to be affected by FoxOs)
3. The late activation of ErbB pathway indicates a **positive feedback loop of the Akt signaling** (**Reproduction of the same result**)





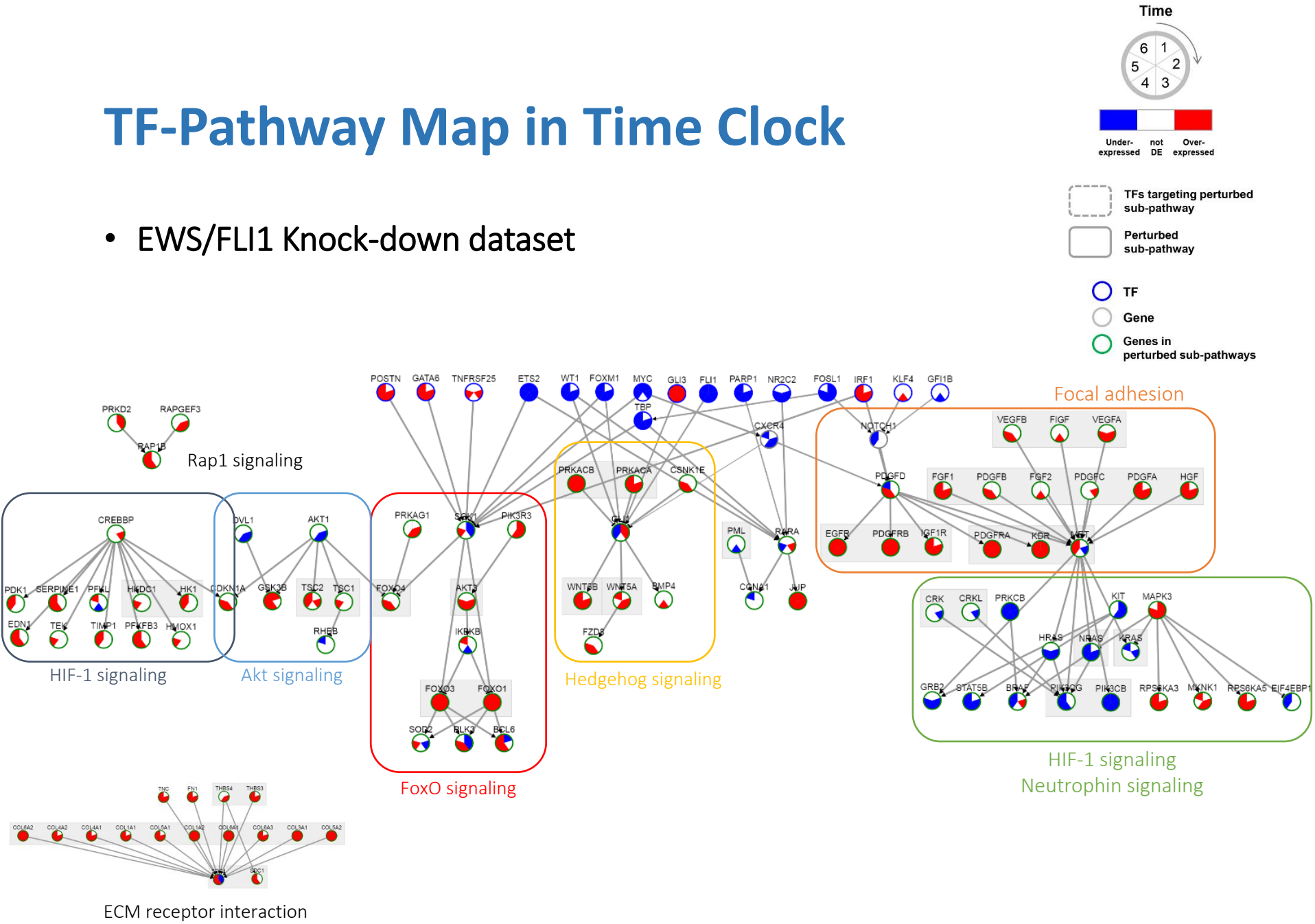
Regulator Analysis Result

TimeTP		MRA		DREM
Rank	TF	Rank	TF	TF
1	NKX3-1	1	SREBF1	FOXF2, NF1, SRF
2	LMO2			
3	ATF3			
4	FOXA1			
5	CEBPA			
6	FOXO4			
7	FOXL1			
8	RFX1			
9	**TGIF1**			
10	SREBF1			
11	FOXO3			
12	USF2			
13	**USF1**			
14	GTF2A1			
15	RORA			
16	**RREB1**			

- TFs predicted and ranked by TimeTP **include three important TFs from the original paper** of the dataset **(Reproduction of the same result)**.

TF-Pathway Map in Time Clock

- EWS/FLI1 Knock-down dataset

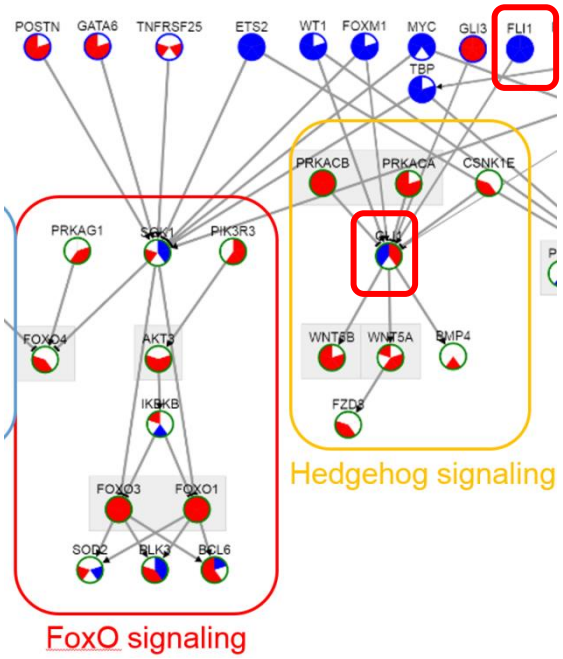


TF-Pathway Map in Time Clock

- **Findings**
  - **Perturbation in cancer-related pathways**
    - Signaling pathways
    - Adhesion-related pathways
      (known target of EWS/FLI1)

  - **Major findings supported by the previous studies:**

1. **FLI1 (EWS/FLI1)**
   (Reproduction of the same result)
2. **GLI1** (a central mediator of EWS/FLI1 signaling in Ewing tumors)
3. **FOXO1** (Known target of EWS/FLI1) and downstream pathways

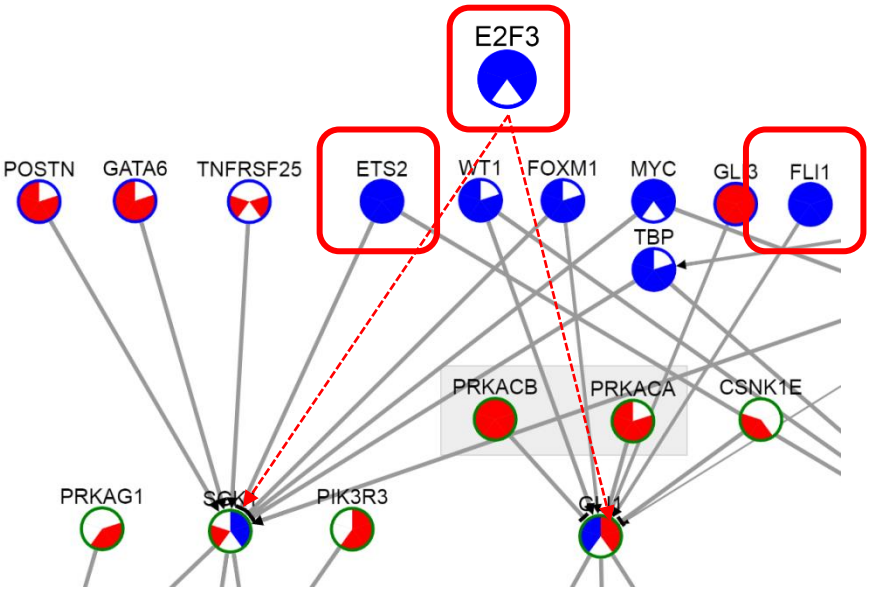


TF-Pathway Map in Time Clock

- **Findings**

  4. When E2F3 is added in a network, E2F3 is predicted to target SGK1 and GLI1 (same as FLI1 or ETS2)

  - Indicating **E2F3 co-regulation with EWS/FLI1**
    (Reproduction of the same result)



Summary

Cross-correlation	Estimates expression delay between two genes to find sub-pathways
Influence maximization	Finding and ranking TFs regulating sub-pathway genes
TF-Pathway map in time clock	Visualization of pathway perturbation dynamics

http://biohealth.snu.ac.kr/software/TimeTP

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TimesVector: A vectorized clustering approach to the analysis of time series transcriptome data from multiple phenotypes

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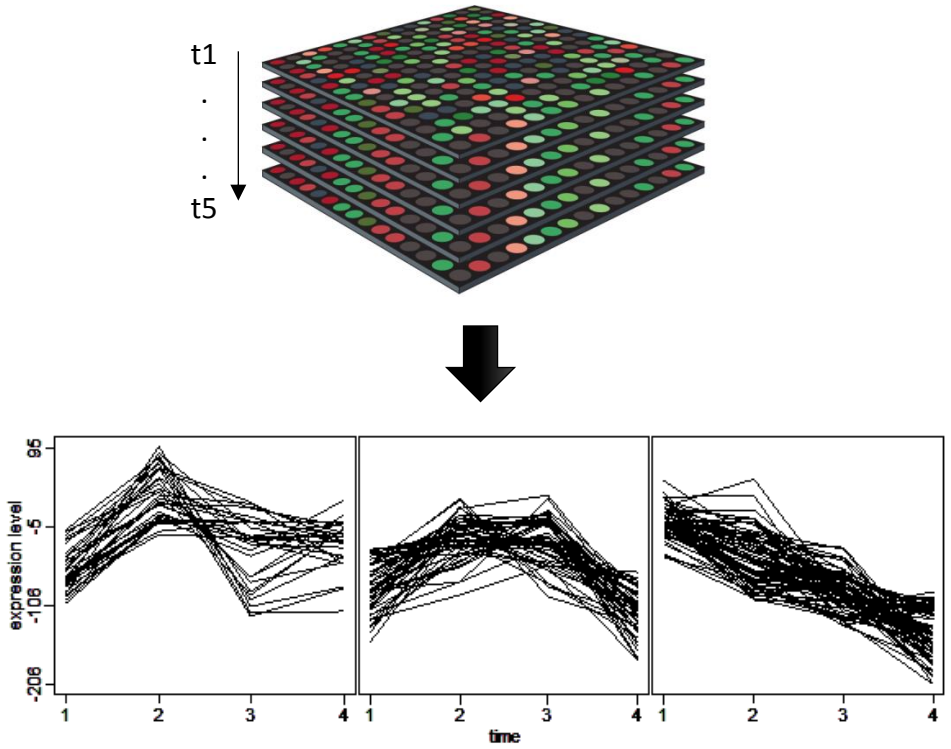
Time-Series clustering

- Identifying a set of genes with a similar expression pattern can reveal co-regulated genes under a condition of interest (e.g., stress, developmental phase, phenotype difference)



Goal of this study

Why?

Compare multiple time series data with each data being sampled at **different conditions** (i.e., experimental conditions, phenotypes) to identify similar and different biological mechanisms

How?

Identify biologically meaningful **gene clusters** (**triclusters**) that have significantly similar or different expression patterns from **3 dimensional time series data** (Gene-Time-Condition)

Backgrounds:
Methods for time series gene clustering

- Clustering methods can be classified by the dimension of the time series data

- **1 Dimensional** – Single time point (static) gene expression data

- **2 Dimensional** – Multiple time points gene expression data

- **3 Dimensional** – Multiple time points and conditions gene expression data

Backgrounds:
One Dimensional (time series) clustering analysis

- Differentially expressed analysis is done for 1D data
  - Differential expressed genes are required to have a gene expression fold change above a threshold in at least to consecutive time points (Nau G. et al. PNAS 2002, Shapira S. et al. Cell 2009)

- Time series DEG tools
  - Significance Analysis of Microarrays (SAM), Tusher et al. PNAS (2001)
  - Bayesian Estimation of Temporal Regulation (BETR), Aryee et al. BMC Bioinformatics (2009)

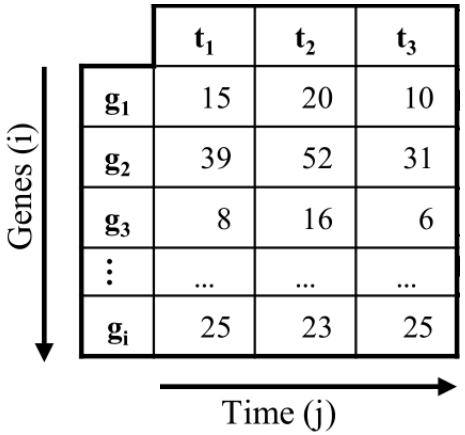
- Drawbacks
  - Heuristic approach
  - Does not take into account the continuous nature of time series data (independent statistical test on each time point)

Backgrounds:
Two Dimensional (time series) clustering analysis

- A number of methods were proposed for clustering 2D time series data among which **biclustering** approaches were most successful

- Biclustering is able to identify genes with **similar expression patterns** across the time dimension. Since it performs **sub-space clustering**, it is able to detect similar expression with time lag.
  - Cheng and Church, ISMB (2000)
  - BiGGEsTS (Biclustering Gene Expression Time Series), Joana P. BMC Research Notes (2009)

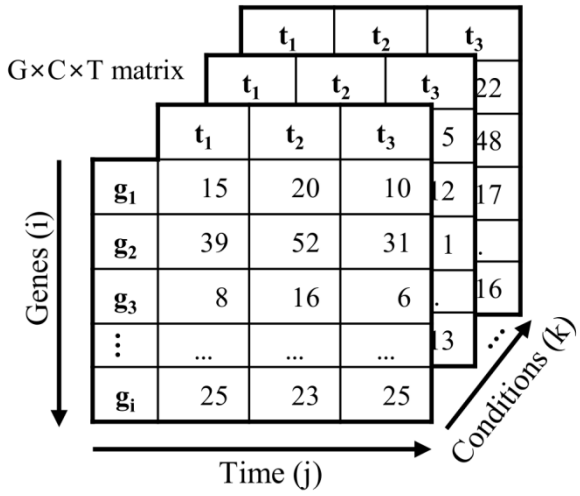
- Drawbacks
  - Biclustering is **NP hard**, hence relies on heuristic methods or probabilistic approximation
  - Cannot detect clusters with different expression patterns



	t_1	t_2	t_3
g_1	15	20	10
g_2	39	52	31
g_3	8	16	6
⋮	...	...	...
g_i	25	23	25

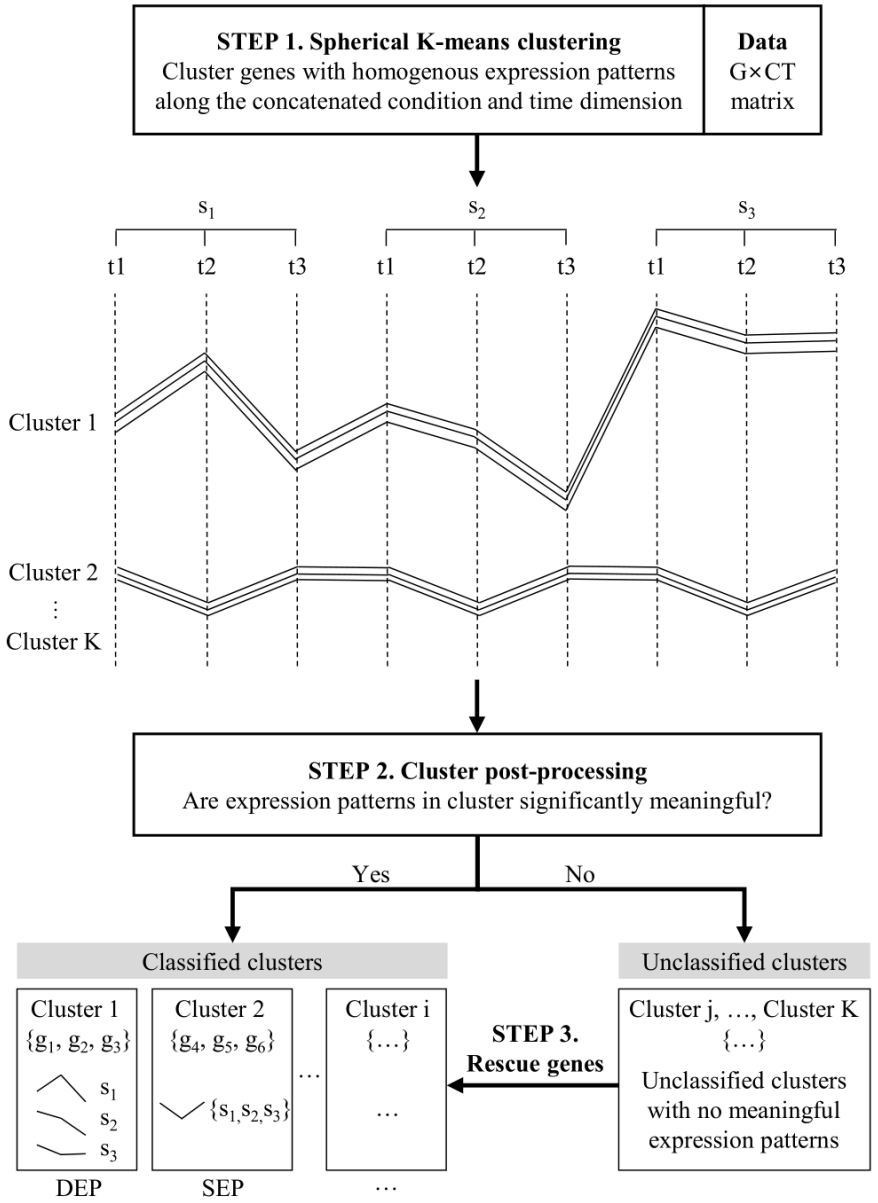
Backgrounds:
Three Dimensional time series analysis

- Three dimensional data constitutes **G**enes, **T**ime points and **C**onditions
  - Conditions refers to the different condition of each time series data (i.e., phenotype, environment stress, experimental design)
- Clustering such 3D data is known as triclustering
- Only a few triclustering algorithms are present
  - TriCluster (Zhao and Zaki, 2005)
  - OPTricluster (Tchagang 2012)
- Drawbacks
  - TriCluster performs biclustering on each time slice (not free from NP hard problem)
  - OPTricluster cannot detect clusters with different expression patterns across all conditions



TimesVector

- A **triclustering** algorithm that is able to cluster genes with **similar or different** expression patterns among multiple conditions from 3D time series data



Motivation

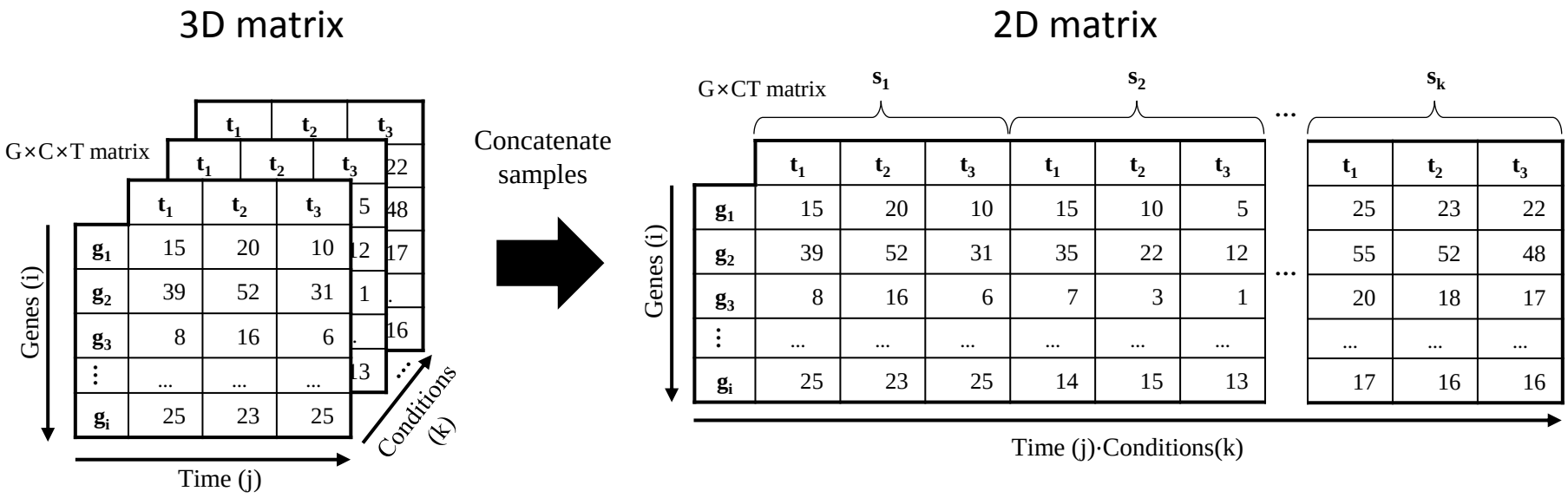
Tow major challenges in Triclustering

1. Clustering high dimensional data is difficult
   (2D biclustering is already NP-hard)

2. Detecting triclusters where the expression patterns among conditions differ is a non-trivial problem

Solving 1st challenge – Curse of dimensionality

- **Dimension reduction** by stripping away the sample dimension and concatenating it to the time dimension
- Takes burden off of for clustering and post-processing procedures



Clustering genes

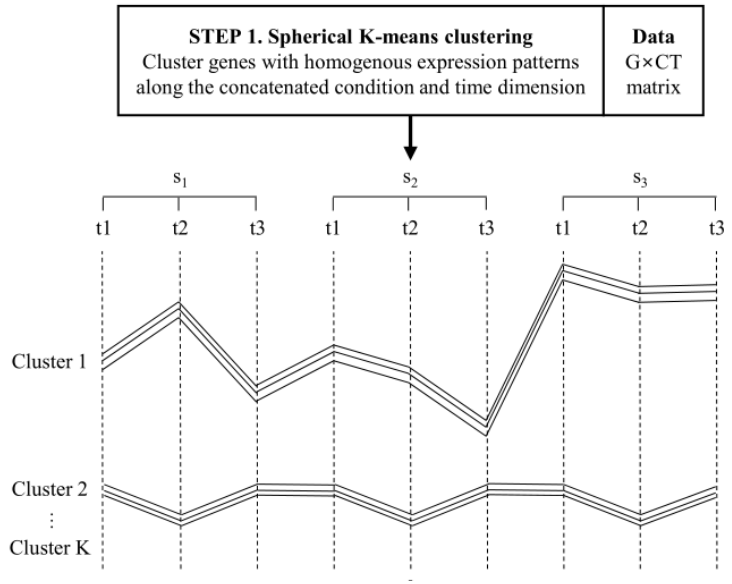
Perform Spherical K-means clustering on the G×CT data matrix

- Objective function is to minimize the cosine distance between the genes and the centroid of the cluster

$$\sum_{i}^{n}\sum_{j}^{k}\mu_{ij}(1-\cos(g_i,c_j))$$

n=genes, k=clusters, μ=membership of gene to cluster, g=gene, c=centroid

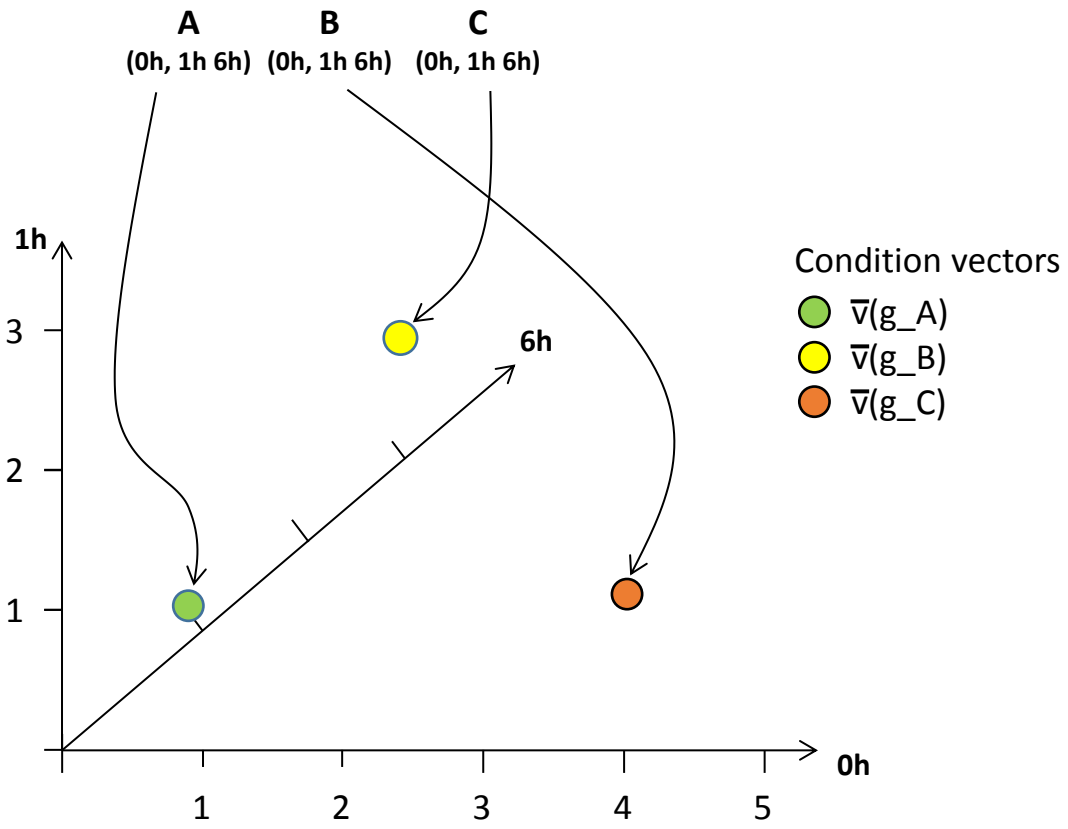
Genes with similar expression patterns across the CT dimension will be clustered together



Solving 2nd challenge – Detecting cluster patterns

- **Re-introduce Sample dimension** by splitting vector in sample domain
- Each gene vector is dissected according to the number of conditions

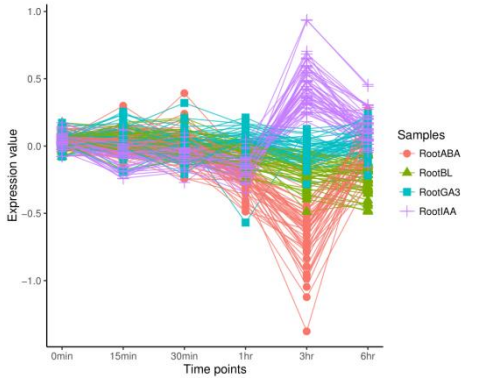
$\bar{v}(g)=\langle 1, 1, 1, 4, 1, 2, 2.5, 3, 3\rangle$



Solving 2nd challenge – Detecting cluster patterns

DEP (Differentially Expressed Pattern)

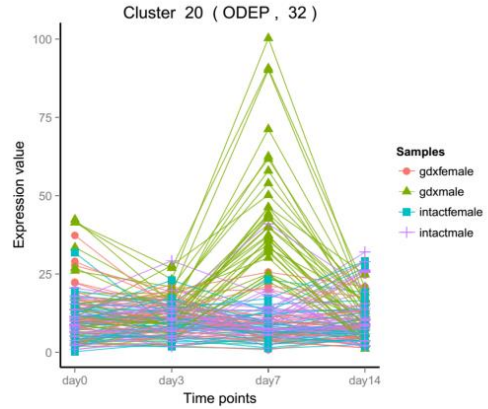
- All samples in a cluster have different expression patterns



Compute **mutual information** to see if the pattern of each condition correlates well with the condition label

ODEP (One Differentially Expressed Pattern)

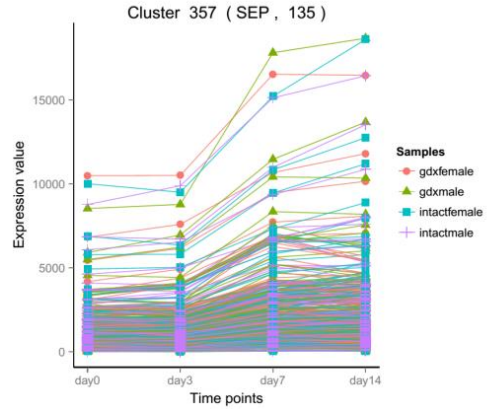
- One sample in a cluster have different expression from the others



Perform **ANOVA** on the condition vector's cosine distance to see if one pattern is significantly different

SEP (Similarly Expressed Pattern)

- All samples have similar expression pattern in a cluster



Statistically test if within cluster cosine distance is significantly **tight** (i.e., pattern is similar)

Rescuing genes

K-means suffers from the problem of pre-defined K clustering

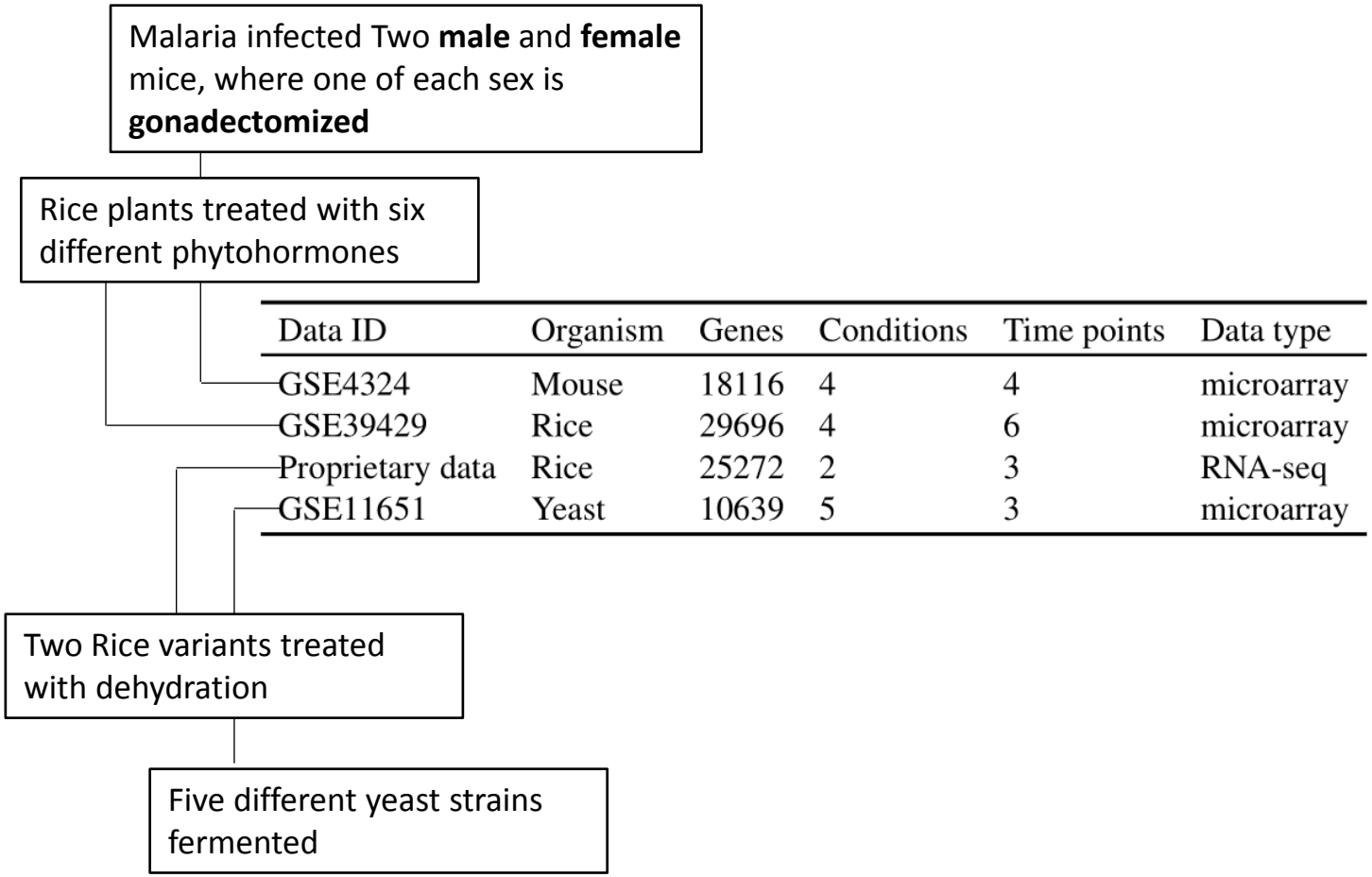
Hence, some genes may be wrongly clustered, which cluster is rejected due to insignificance

Each gene in rejected clusters are reassigned to accepted clusters and rescued if the fitness of the cluster improves

- Approximately 2.5% of rejected genes were rescued in average

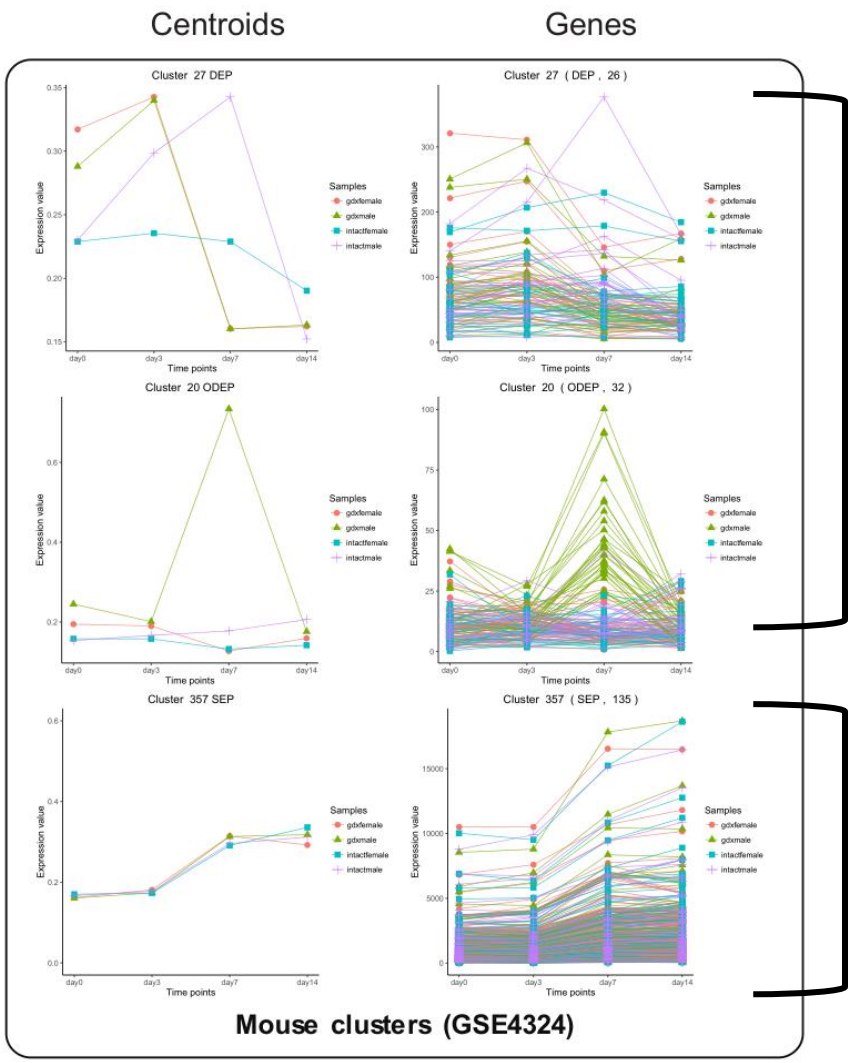
Experiments

TimesVector was performed on 4 time series data sets, each with a different number of genes, time points and conditions



Data ID	Organism	Genes	Conditions	Time points	Data type
GSE4324	Mouse	18116	4	4	microarray
GSE39429	Rice	29696	4	6	microarray
Proprietary data	Rice	25272	2	3	RNA-seq
GSE11651	Yeast	10639	5	3	microarray

Clustering Results – Mouse Data (GSE4324)



Goal of study: Identify genes that are caused by sex difference and respond to gonadectomized conditions

Pathways	Genes	p-value
Alzheimer disease-presenilin	24	6.19×10^{-04}
Cadherin signaling	24	5.63×10^{-03}
Axon guidance mediated by Slit/Robo	9	6.95×10^{-03}
Wnt signaling	37	1.00×10^{-02}
Gonadotropin releasing hormone receptor	32	1.51×10^{-02}
TGF-beta signaling	17	3.83×10^{-02}

The significant pathways detected in genes of **DEP** and **ODEP** clusters are previously reported to be related to malaria infection.

The significant pathways detected in genes of **SEP** clusters were related to responsive signals to infection, such as “T cell activation” and “B cell activation”.

Clustering Results – RiceData (GSE39429)

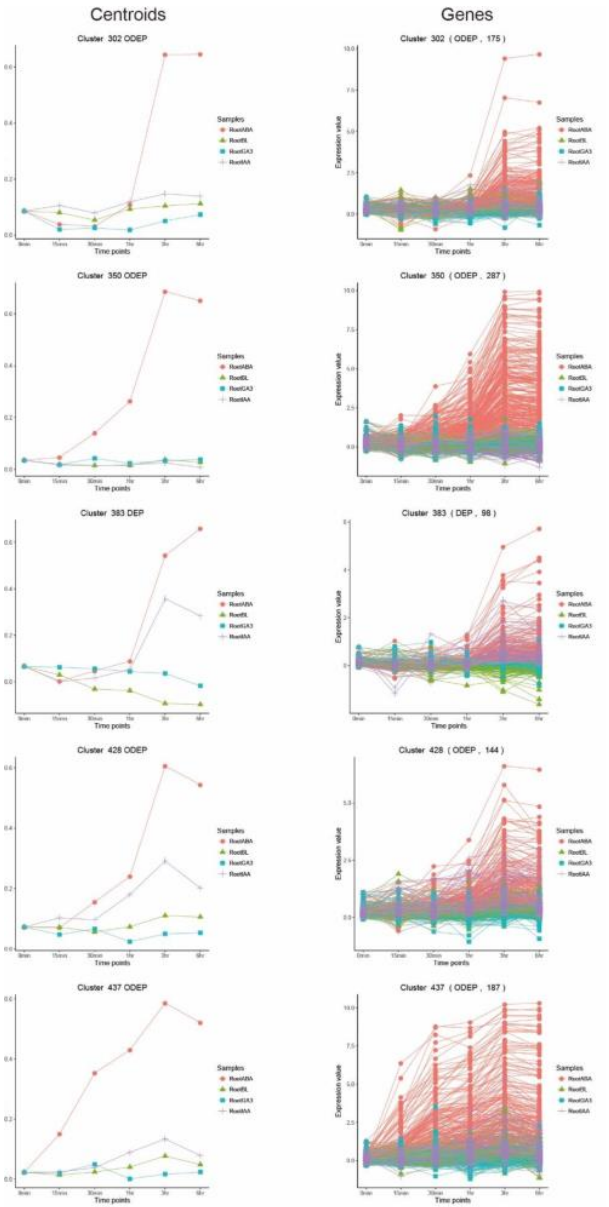
Cluster 302
(175 genes)

Cluster 350
(287 genes)

Cluster 383
(98 genes)

Cluster 482
(144 genes)

Cluster 437
(187 genes)

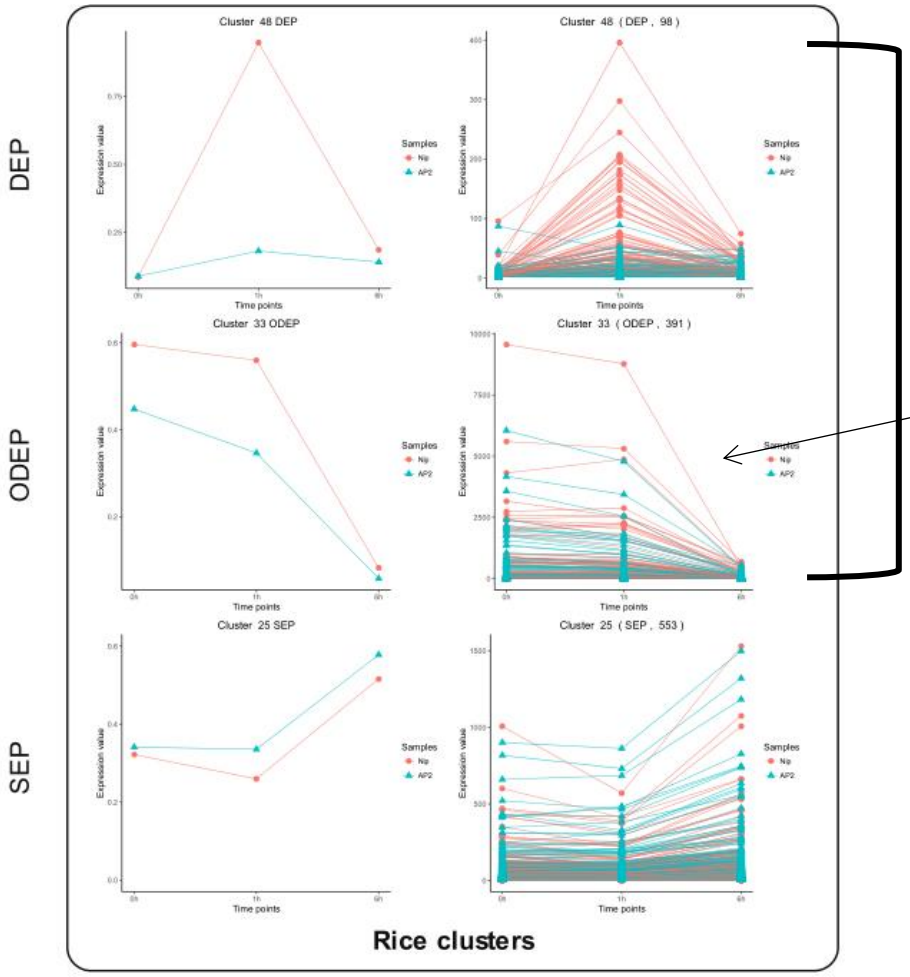


Goal of study: Identify genes that respond to specific phytohormones
(Abscisic acid, Gibberellin, Auxin, Brassinosteroid, Cytokinin and Jasmonic acid)

Among the phytohormones, we found **5 clusters** that strongly respond to **Abscisic acid (ABA)**. The clusters also show gradual up-regulation of genes at different time points.

These up-regulated genes are significanlty related to “aging” , “organ senscence” and “response to hydrogen peroxide” GO terms, which are known responses to ABA.

Clustering Results – Rice Data (Proprietary data)

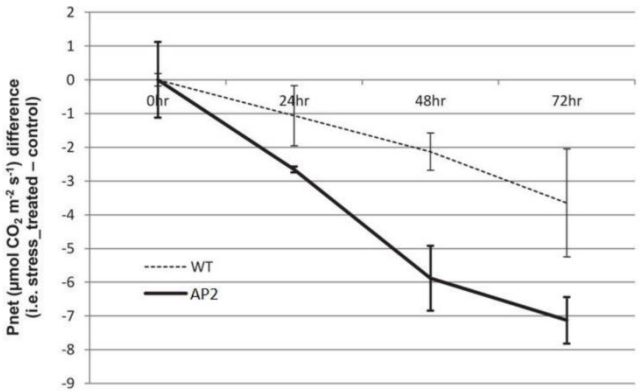


Goal of study: Identify genes that are differentially expressed in drought susceptible and drought tolerance rice plants

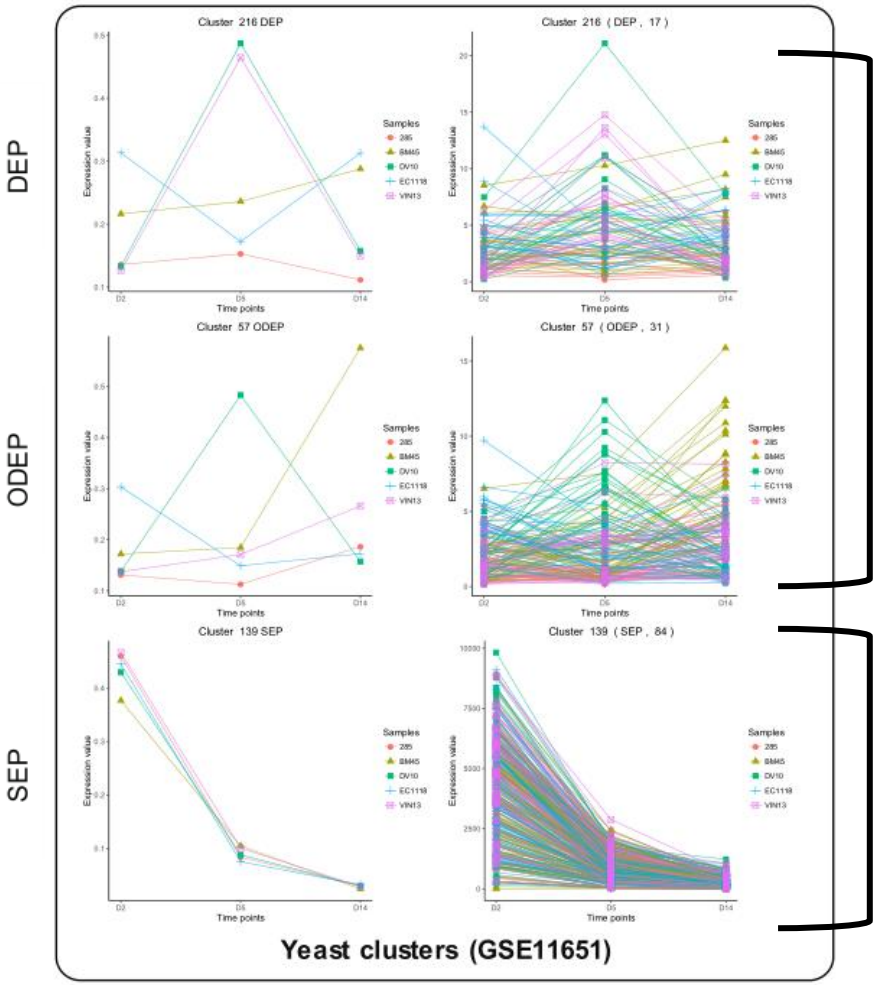
The significant GO terms detected in DEP/ODEP clusters were “Photosynthesis” and “Light harvesting”. Photosynthesis related genes were mainly found in **cluster 33** (**red**: WT, **blue**: AP2 transgenic plant).

Photosynthesis related genes were down-regulated in both plants but greater in AP2.

Experimental validation of net photosynthesis



Clustering Results – Yeast Data (GSE11651)



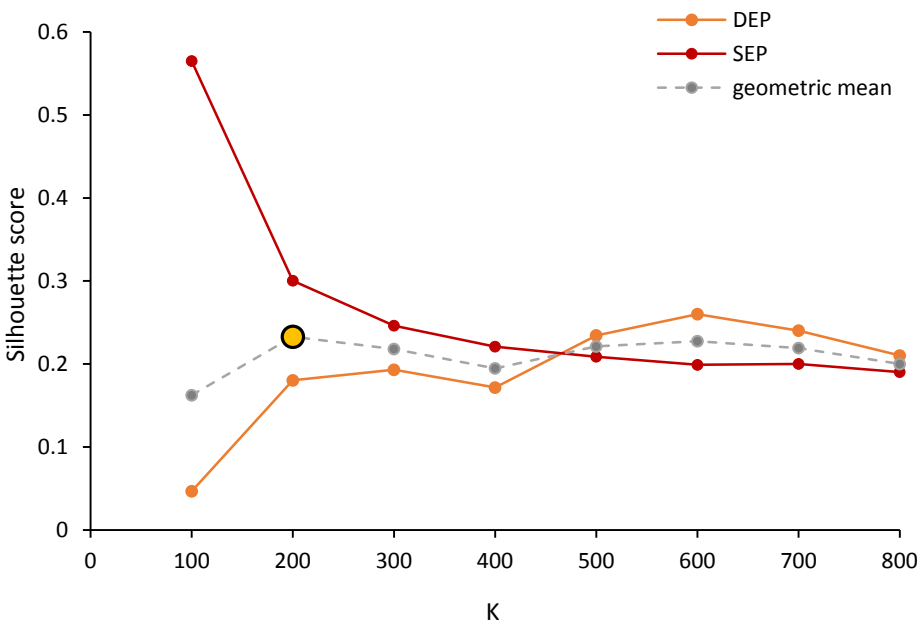
Goal of study: Identify genes that are differentially expressed to specific yeast strains during fermentation to find target genes for metabolic engineering of aromatic compounds

Aromatic compound GO terms were significantly enriched in **DEP/ODEP** clusters. This was reported in the study that generated this data.

The related study reported that the fermentation kinetics were all similar in five strains, which is well reflected in **SEP** clusters

Selecting optimal K

Run TimesVector over a range of K and select K with maximum silhouette score (geometric mean of DEP and SEP silhouette score)



Testing with several data sets we found that K may be set as

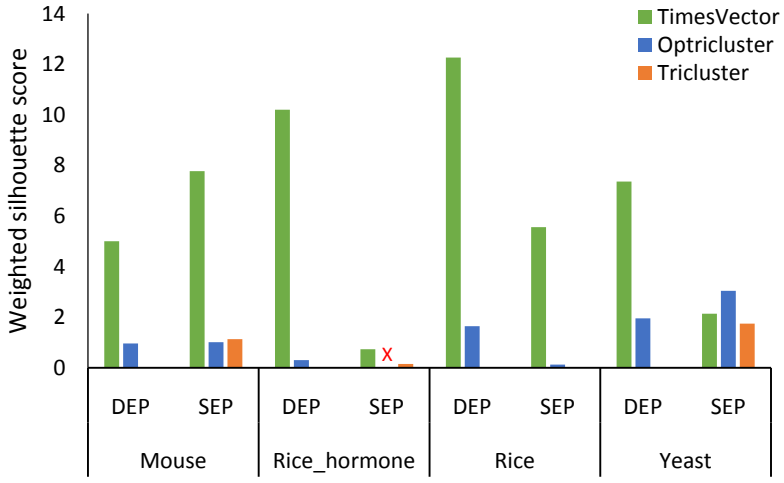
$$K = -85.71 + 28.57(C \times T)$$

E.g.) A time series data with 3 time points and 5 conditions, K=200 (rounded)

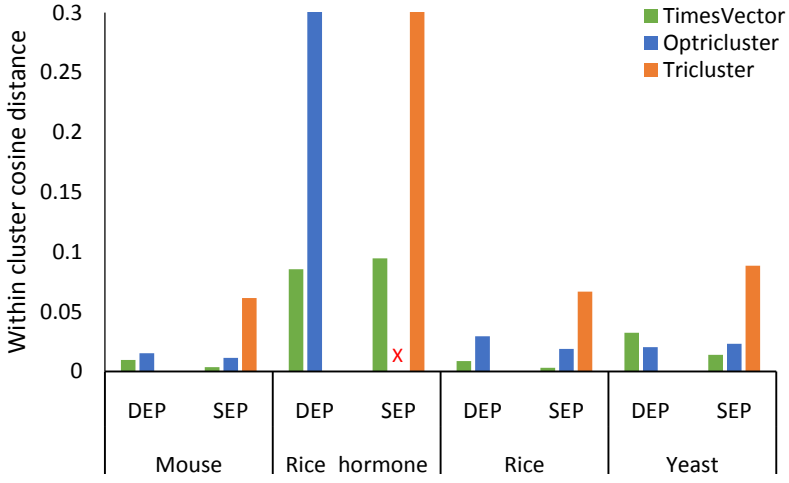
Comparing clustering performance

Compare the clustering results of TimesVector with TriCluster and OPTricluster in terms of weighted silhouette score and within cluster cosine distance

Testing how well clusters are separated



Testing tightness of cluster



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Lab Members (2016 Day1)

감사합니다.

Thank you!