HGC スパコン Web サービス利用法講習会
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大規模遺伝子ネットワーク推定ソフトウェア SiGN

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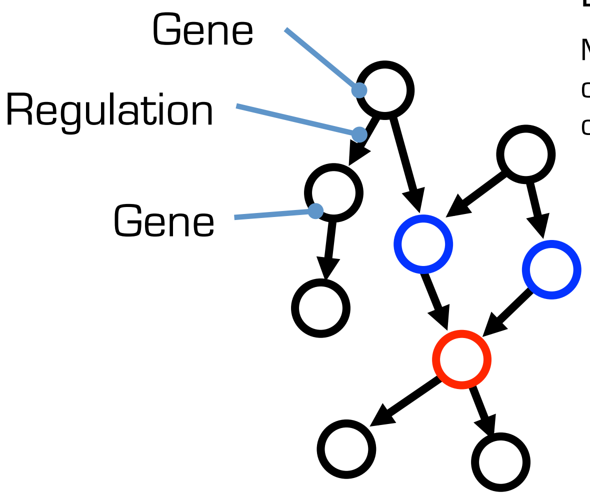
本日の内容

- 遺伝子ネットワーク推定ソフトウェアSiGN（サイン）の紹介
  - SiGN-SSM, SiGN-BN, SiGN-L1

- ベイジアンネットワークを用いたSiGN-BNの機能紹介

Gene Network Estimation

- **Gene network**: model for **transcriptome level gene–gene regulation** using directed graphs.
- Gene network estimation is to estimate gene networks from **high-throughput biological data** e.g. **gene expression data**.



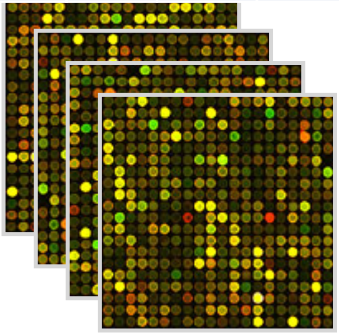
Gene Network

Directed Graph:

Mathematical model consisting of nodes and directed edges connecting them.

Estimation

Gene	KD1	KD2	KD3	…
Gene 1	1.45	-1.54	1.23	…
Gene 2	3.21	-2.1	1.44	…
…	…	…	…	…



Gene Expression Data

SiGN （サイン）

- SiGN: A collection of large-scale gene network estimation software designed for utilizing super computers.
  - SiGN-BN: Bayesian networks （ベイジアンネットワーク） 本日ベータ版公開
  - SiGN-SSM: State space models （状態空間モデル） オープンソース公開中
  - SiGN-L1: L1-regularization based models （L1正則化） 準備中

SiGN Web Site: http://sign.hgc.jp/

SiGN　（サイン）

- HGCスパコンで動くスパコン用専用ソフトウェア
- 実行にはHGCのアカウントが必要
- 実行はターミナルアプリから手でコマンド入力
  - 多少の Unix 操作の知識が必要

SiGNでできること（まとめ）

- 遺伝子発現データから遺伝子間の発現の依存関係を予測・推定する
  - 遺伝子発現データ（マイクロアレイデータ）
    - 患者サンプルから得られる細胞
    - ノックダウン実験
    - 薬剤投与などの時系列に観測したデータ
- ある程度のサンプル数が必要
  - 必要な量はモデルや実験デザインにより様々

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    （L1正則化）

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SiGN-SSM

Dynamic gene network estimation software using a State Space Model (SSM).

（状態空間モデル）

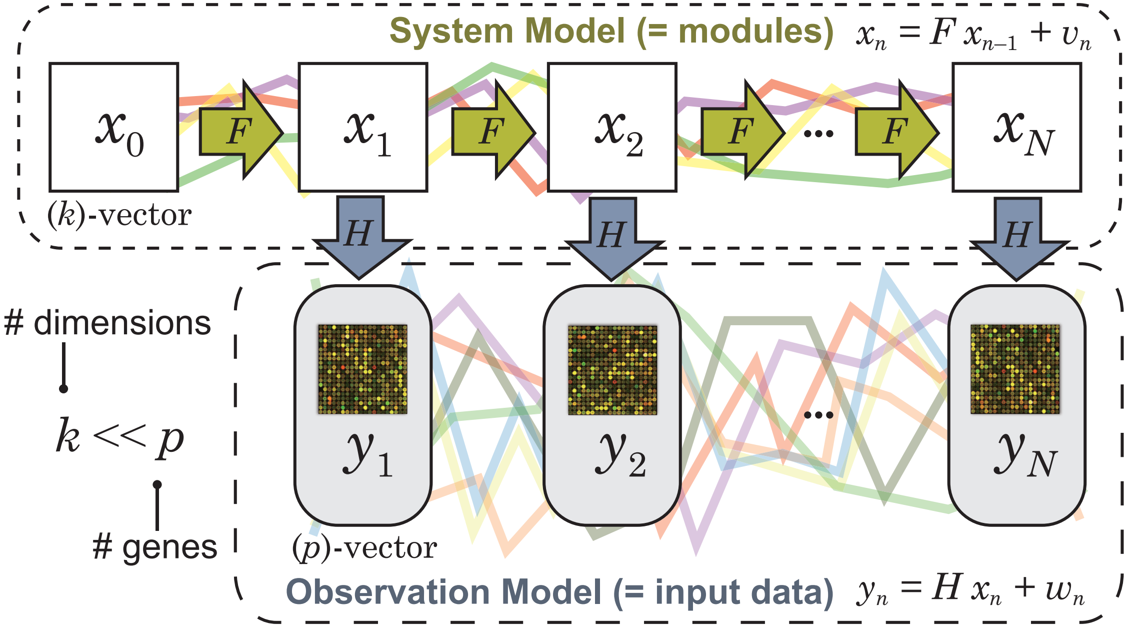
Open source software distributed at http://sign.hgc.jp/signssm/

Suitable for modeling time series gene expression data.

Definition:

$$x_n = Fx_{n-1} + v_n, \quad v_n \sim N(0,Q) \qquad \text{[System model]}$$

$$y_n = Hx_n + w_n, \quad w_n \sim N(0,R) \qquad \text{[Observation model]}$$



Parameter estimation = EM algorithm (x_0, F, Q, H, R and k)

Needs to repeat estimation with various initial values.

SiGN-SSM: 特徴

- 時系列マイクロアレイデータからの遺伝子ネットワーク推定

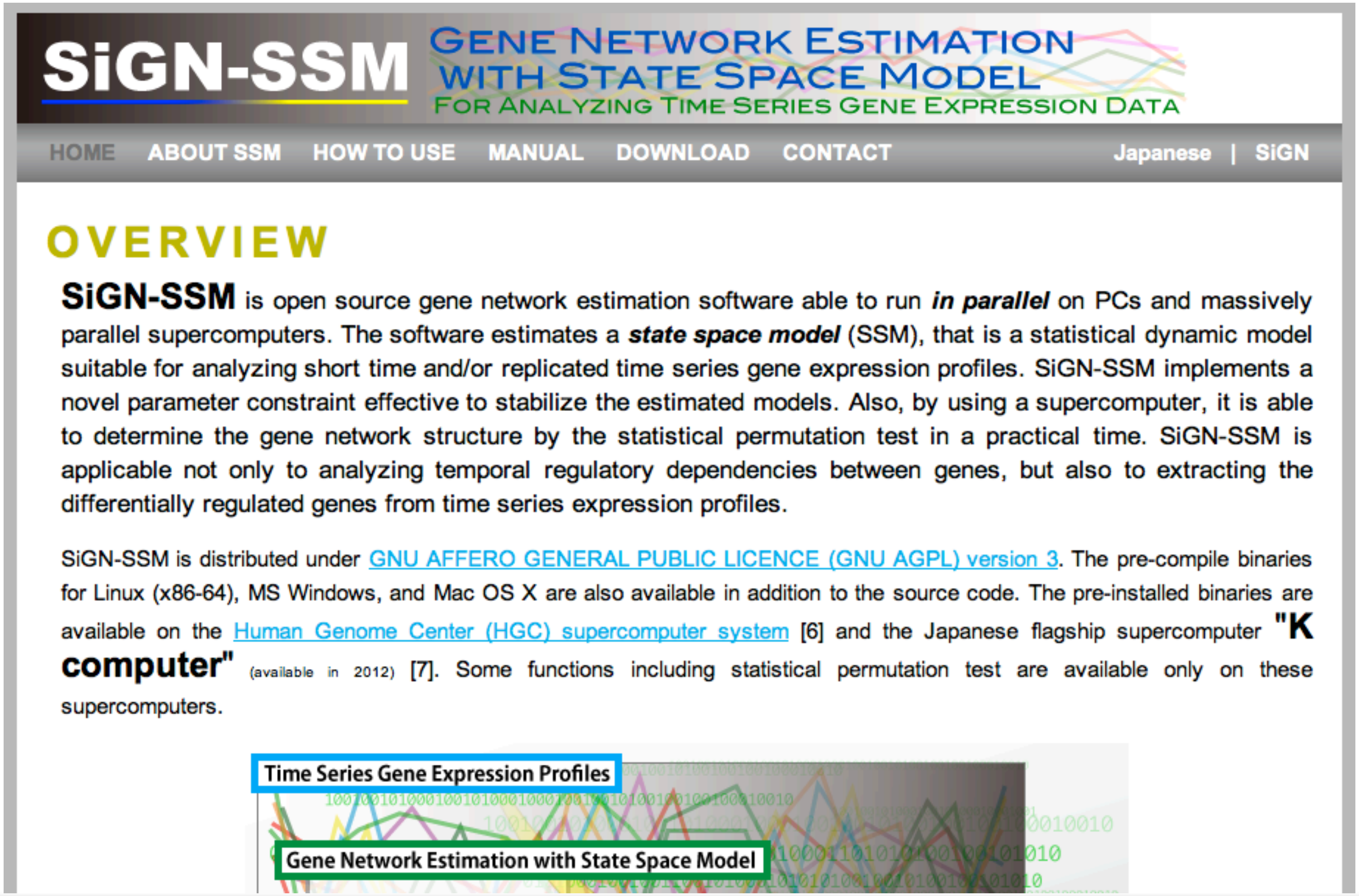
- 不等間隔・繰り返し計測データに対応

- SSMのパラメータ推定.
- 推定結果に基づきネットワーク構造の決定.

- オープンソース
  - 一部機能はHGCスパコン限定

SiGN-SSM: 詳細はウェブサイトへ

http://sign.hgc.jp/signssm/


SiGN-SSM
GENE NETWORK ESTIMATION WITH STATE SPACE MODEL
FOR ANALYZING TIME SERIES GENE EXPRESSION DATA
HOME
ABOUT SSM
HOW TO USE
MANUAL
DOWNLOAD
CONTACT
Japanese | SiGN
Time Series Gene Expression Profiles
Gene Network Estimation with State Space Model

OVERVIEW

SiGN-SSM is open source gene network estimation software able to run ***in parallel*** on PCs and massively parallel supercomputers. The software estimates a ***state space model*** (SSM), that is a statistical dynamic model suitable for analyzing short time and/or replicated time series gene expression profiles. SiGN-SSM implements a novel parameter constraint effective to stabilize the estimated models. Also, by using a supercomputer, it is able to determine the gene network structure by the statistical permutation test in a practical time. SiGN-SSM is applicable not only to analyzing temporal regulatory dependencies between genes, but also to extracting the differentially regulated genes from time series expression profiles.

SiGN-SSM is distributed under GNU AFFERO GENERAL PUBLIC LICENCE (GNU AGPL) version 3. The pre-compile binaries for Linux (x86-64), MS Windows, and Mac OS X are also available in addition to the source code. The pre-installed binaries are available on the Human Genome Center (HGC) supercomputer system [6] and the Japanese flagship supercomputer **"K computer"** (available in 2012) [7]. Some functions including statistical permutation test are available only on these supercomputers.

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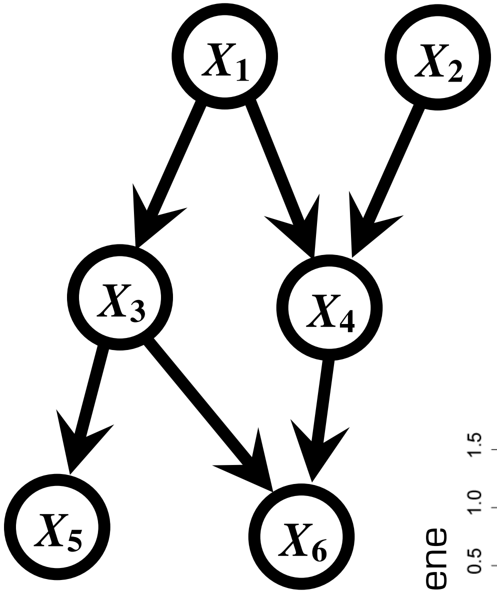
Nonparametric Bayesian Network Model

We use the nonparametric Bayesian network as models for gene networks

Node = Gene



Directed Edge = Regulatory Relationships

Joint Probability by a DAG (Directed Acyclic Graph)

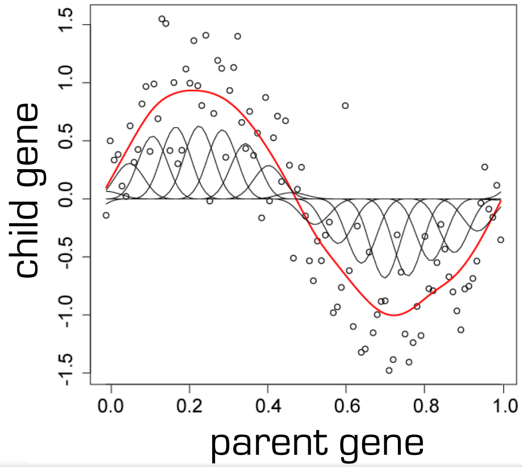
$$f(X_1,X_2,\ldots,X_6)$$

$$= f_1(X_1)f_2(X_2)f_3(X_3\mid X_1)\cdots f_6(X_6\mid X_3,X_4)$$

Network score = Posterior Probability

$$\pi(G\mid X)\propto \pi(G)\int\prod_{i=1}^{n} f(x_{i1},\ldots,x_{ip}\mid \boldsymbol{\theta}_G)\pi(\boldsymbol{\theta}_G\mid\lambda)d\boldsymbol{\theta}_G$$

G : gene network $\quad$ X : expression data



Nonparametric regression by B-spline

$$x_{ij}=m_{j1}(p_{i1}^{(j)})+\ldots+m_{jq_j}(p_{iq_j}^{(j)})+\varepsilon_{ij}$$

$$\varepsilon_{ij}\sim N(0,\sigma_j^2)$$

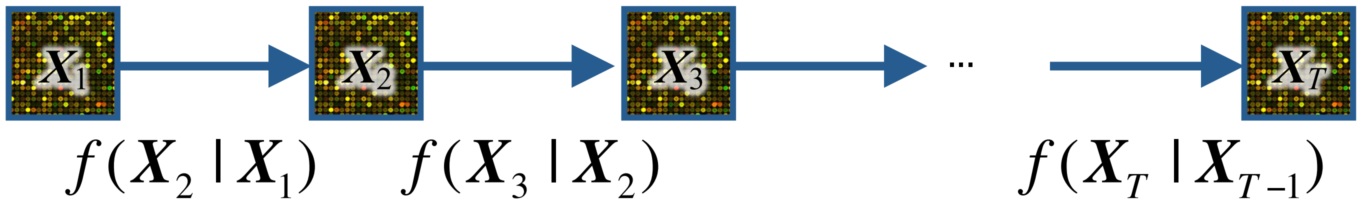
$$m_{jk}(p_{ik}^{(j)})=\sum_{l=1}^{M_{jk}}\gamma_{lk}b_{lk}^{(j)}(p_{ik}^{(j)})$$

Dynamic Bayesian Networks

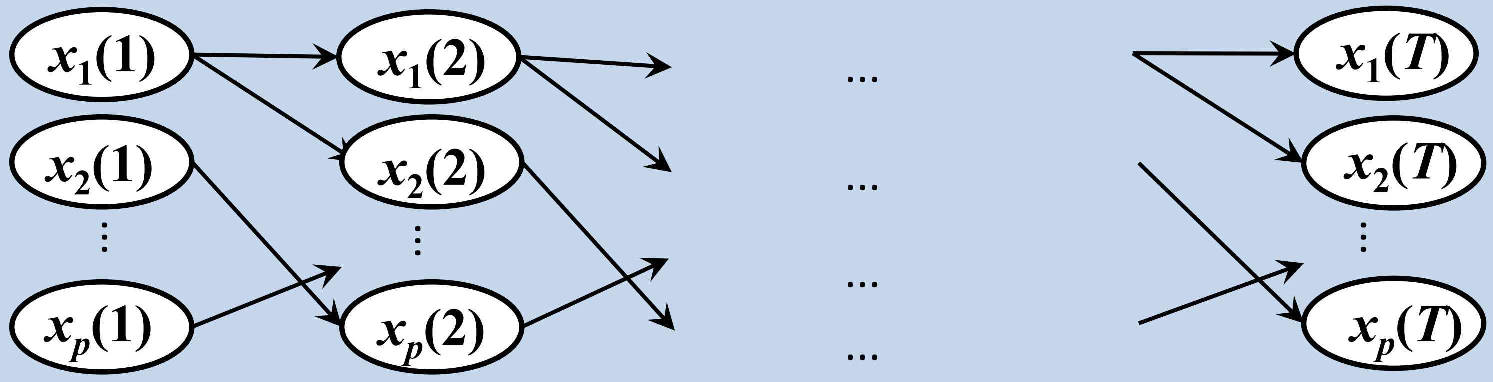
DBN (Dynamic Bayesian Network): model for time course data.

DBN assumes the dependency between consecutive time points.

Time course gene expression data: $X_1, \ldots, X_T$



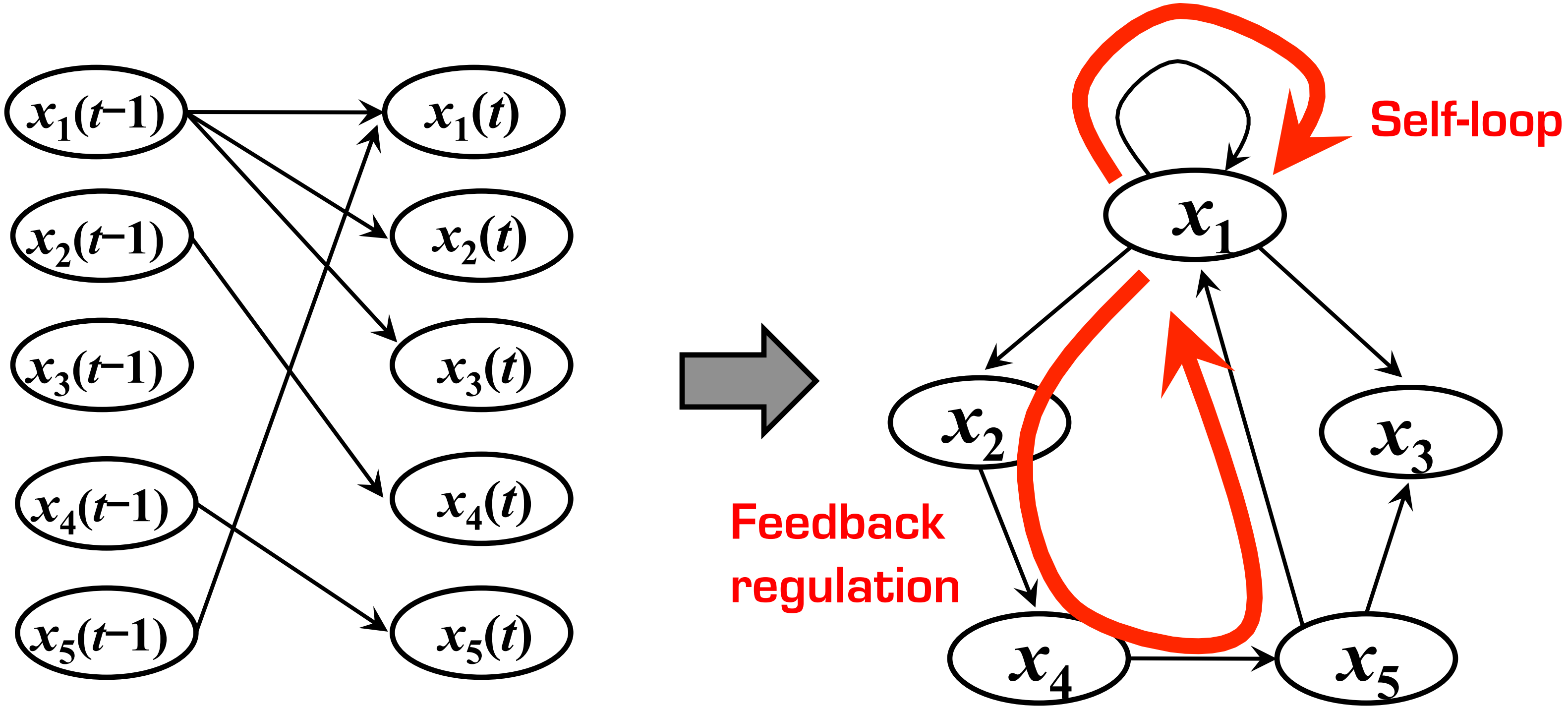
Gene Network: Bipartite Graph



Gene Network Estimation by Dynamic Bayesian Networks

Estimated Bipartite Graph

Corresponding Gene Network



Self-loop

Feedback regulation

Difficulty in Bayesian Network Estimation

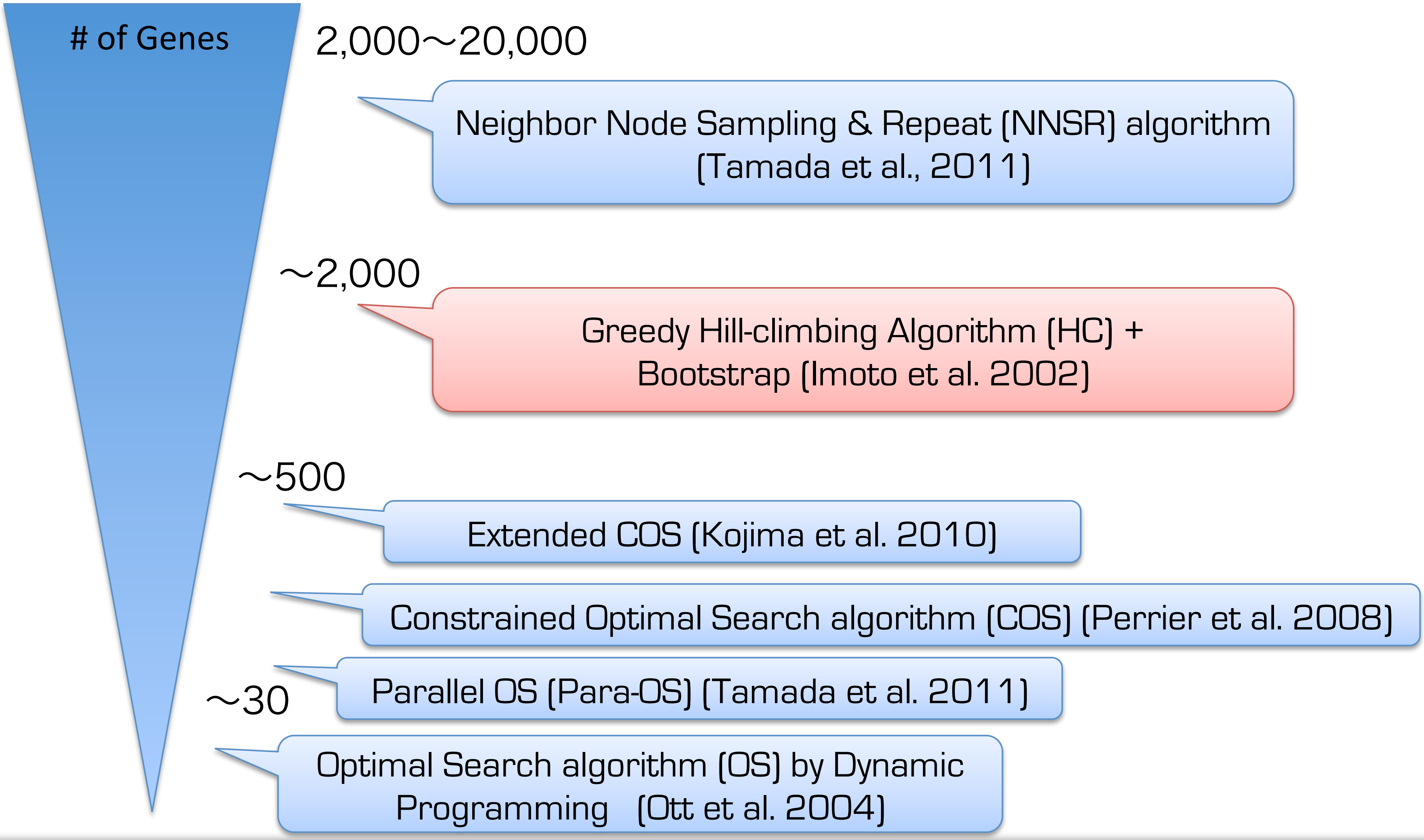
A huge number of possible DAGs : impossible to search the optimal one

# of nodes	# of DAGs	# of nodes	# of DAGs
1	1	16	$\approx 8.37 \times 10^{46}$
2	3	17	$\approx 6.26 \times 10^{52}$
3	25	18	$\approx 9.93 \times 10^{58}$
4	543	19	$\approx 3.32 \times 10^{65}$
5	29,281	20	$\approx 2.34 \times 10^{72}$
6	3,781,503	21	$\approx 3.46 \times 10^{79}$
7	1,138,779,265	22	$\approx 1.07 \times 10^{87}$
8	783,702,329,343	23	$\approx 6.97 \times 10^{94}$
9	1,213,442,454,842,881	24	$\approx 9.43 \times 10^{102}$
10	$\approx 4.17 \times 10^{18}$	25	$\approx 1.86 \times 10^{111}$
11	$\approx 3.15 \times 10^{22}$	⋯	⋯
12	$\approx 5.21 \times 10^{26}$	30	$\approx 2.71 \times 10^{158}$
13	$\approx 1.86 \times 10^{31}$	⋯	⋯
14	$\approx 1.43 \times 10^{36}$	⋯	⋯
15	$\approx 2.37 \times 10^{41}$	40	$\approx 1.12 \times 10^{276}$

Exceeds the number of atoms in the universe

Network Size and Algorithms

Different search algorithms are developed depending on the size of networks



# of Genes

2,000〜20,000

Neighbor Node Sampling & Repeat (NNSR) algorithm (Tamada et al., 2011)

〜2,000

Greedy Hill-climbing Algorithm (HC) + Bootstrap (Imoto et al. 2002)

〜500

Extended COS (Kojima et al. 2010)

Constrained Optimal Search algorithm (COS) (Perrier et al. 2008)

Parallel OS (Para-OS) (Tamada et al. 2011)

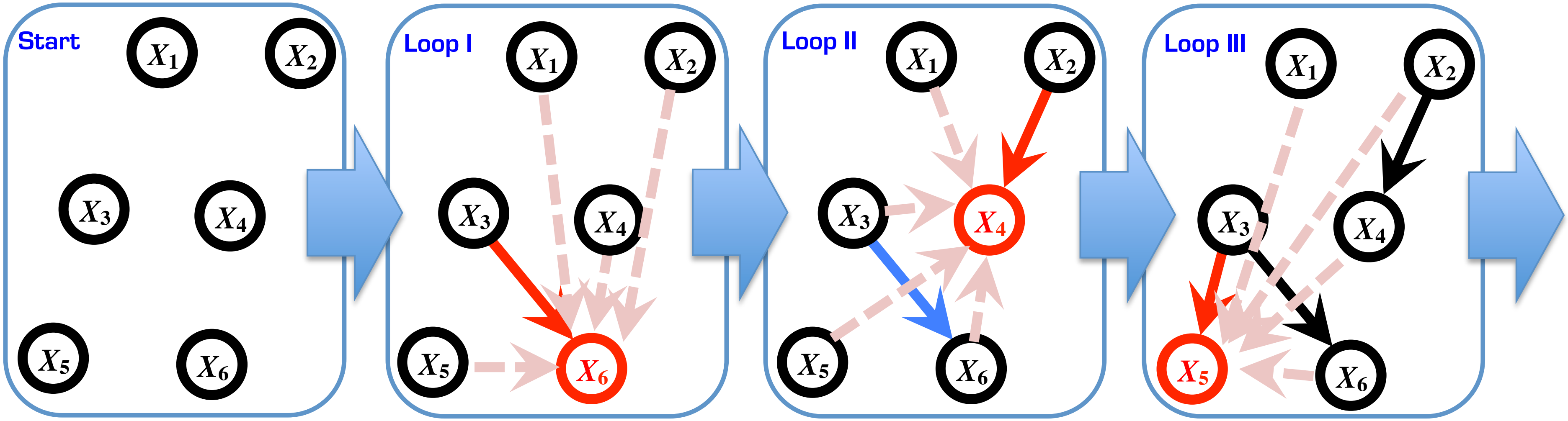
〜30

Optimal Search algorithm (OS) by Dynamic Programming (Ott et al. 2004)

Greedy Hill-Climbing Algorithm (HC)

Algorithm for searching the local optimal DAG structure

Heuristics algorithm applicable to estimate gene networks for ～ **100 genes**.



1. Begins with an empty graph.

2. Visits nodes in a random order.

3. Calculates local scores for all possible candidate parents.

4. Employs the best operation that improves the score.

Add/Delete/Reverse

5. Repeats until any operation can improve the score.

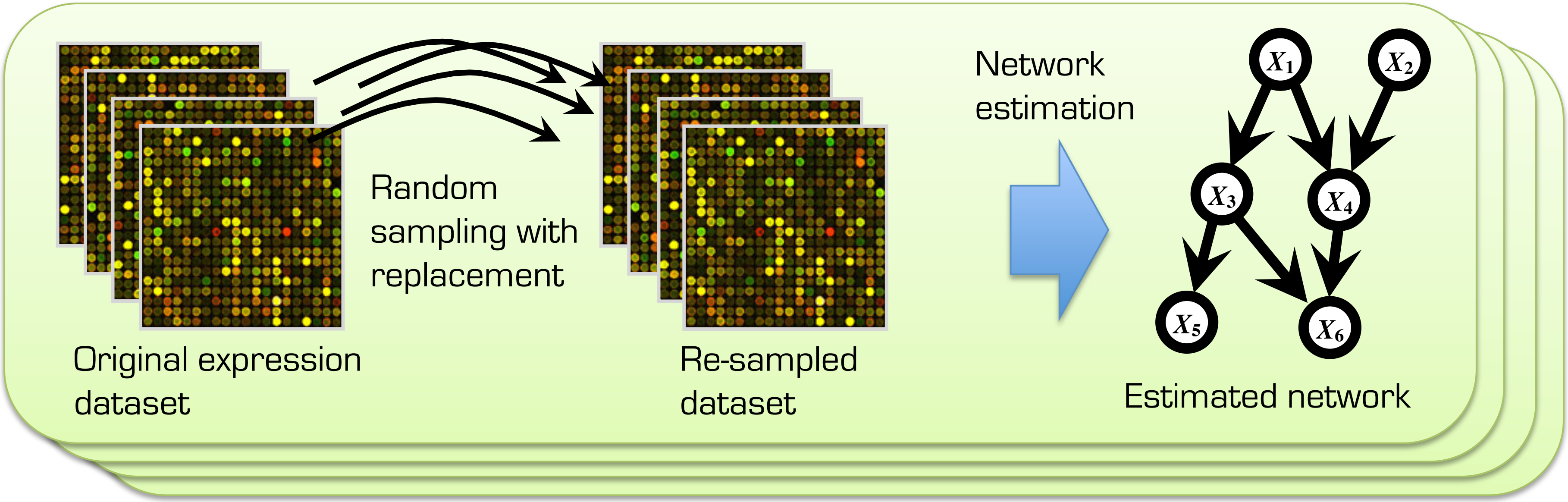
※ Need to check every time whether a cyclic path is made or not.

※ Repeats this many times, then employs the best structure because they are local optimal.

HC + Bootstrap

〜 1000 genes

Bootstrapping is required for calculating the reliability of edges.



Original expression dataset

Random sampling with replacement

Re-sampled dataset

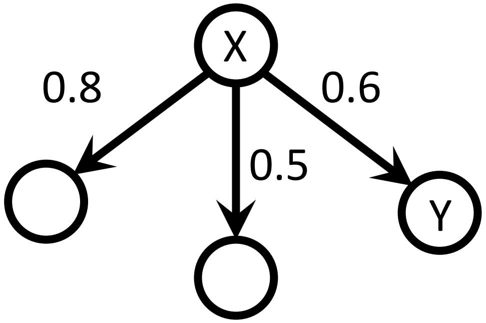
Network estimation

Estimated network

- Estimate networks **many times for re-sampled dataset.** (1,000 times 〜)
- The final structure is determined by the frequencies of edges during the repeated estimation.
- We can perform each network estimation **independently for the re-sampled datasets in parallel.**
  - **Parallelization is easy for Bootstrap HC.**

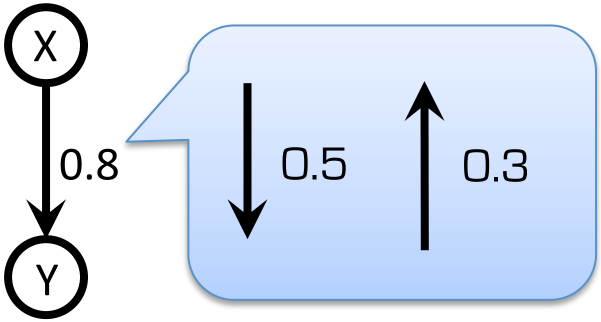
Estimated Networks

Bootstrap probabilities



- Edges in estimated networks have "Bootstrap Probability."

Direction reliabilities (Static model only)



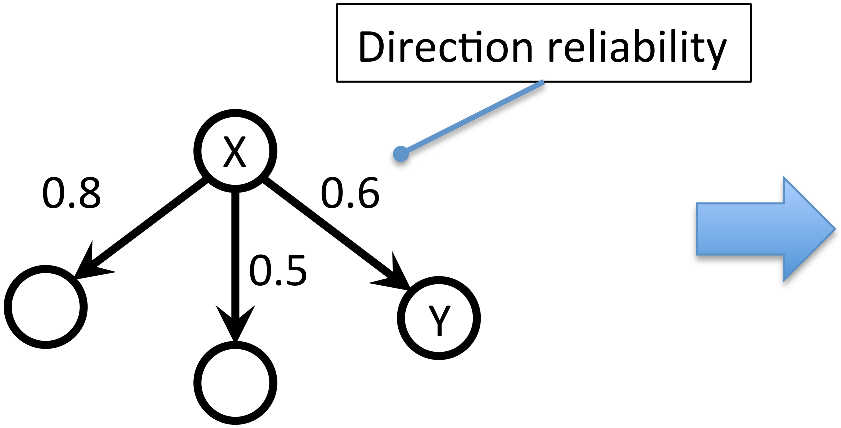
Direction reliability = 0.625

- Edges also have reliability of the direction.
  - Dynamic model can have edges in both directions.

Hubness & Hub Index

Hubness: simply counts the number of children regardless of the confidence of the edge direction.

Hub genes may have important roles as *master regulators* in the networks.
Useful for analyzing the estimated networks.



Hubness of X = 3

Hub Index of X = 1.9

Hubness of Y = 0

Hub Index of Y = 0.4

Hub Index: takes the confidence of edge direction into account. Simply the sum of “BS.Direction” for all edges connected to the target node.

Note: Hub Index does not take bootstrap probabilities into account.

実行・解析の流れ

1. 入力となるアレイデータの準備
   - テキストファイルの入力ファイル
     - EDF, タブ区切り独自形式
2. HGCスパコンへのジョブの投入
   - SGE のアレイジョブによりBootstrapの繰り返し計算を並列実行
3. 出力結果のまとめ処理
   - 結果がファイルに出力されるので，それを１つのネットワークファイルにまとめる
     - CSML, タブ区切りテキストファイル
4. Cell Illustrator Online などによる解析
   - ハブ遺伝子の分析やターゲット遺伝子関連サブネットワークの抽出・分析

SiGN-BN の公開状況

- ベータ版 (rel. 0.9.0) が `~tamada/sign` 以下にインストールされている

- 近日中に正式版およびドキュメントを公開予定
  - HC+Bootstrap 法以外の SiGN-BN のアルゴリズムや SiGN-L1 などども準備が整い次第順次HGCスパコンユーザ向けに公開予定

入力ファイル

- EDF形式によるアレイデータ
- 独自タブ区切りテキストファイル
  - 詳細は後日公開するドキュメントを参照

タブ区切りテキストファイルによる独自フォーマット

Probe_1	Gene_1	0.5	0.3	0.52	…
Probe_2	Gene_2	1.5	1.44	1.55	….
Probe_3	Gene_3	…	…	…	
….	…	…			
Probe_p	Gene_p				

実行

- HGCスパコンにログインし，SGEのqsubコマンドによりジョブを投入・実行

ブートストラップの実行

```
qsub -t 1-X [SGE options] /usr/local/bin/signbn-hc.sh
   --bs -o file_prefix [SiGN options] input_file
```

※ X はブートストラップ回数．1000以上を推奨．

結果のまとめ処理

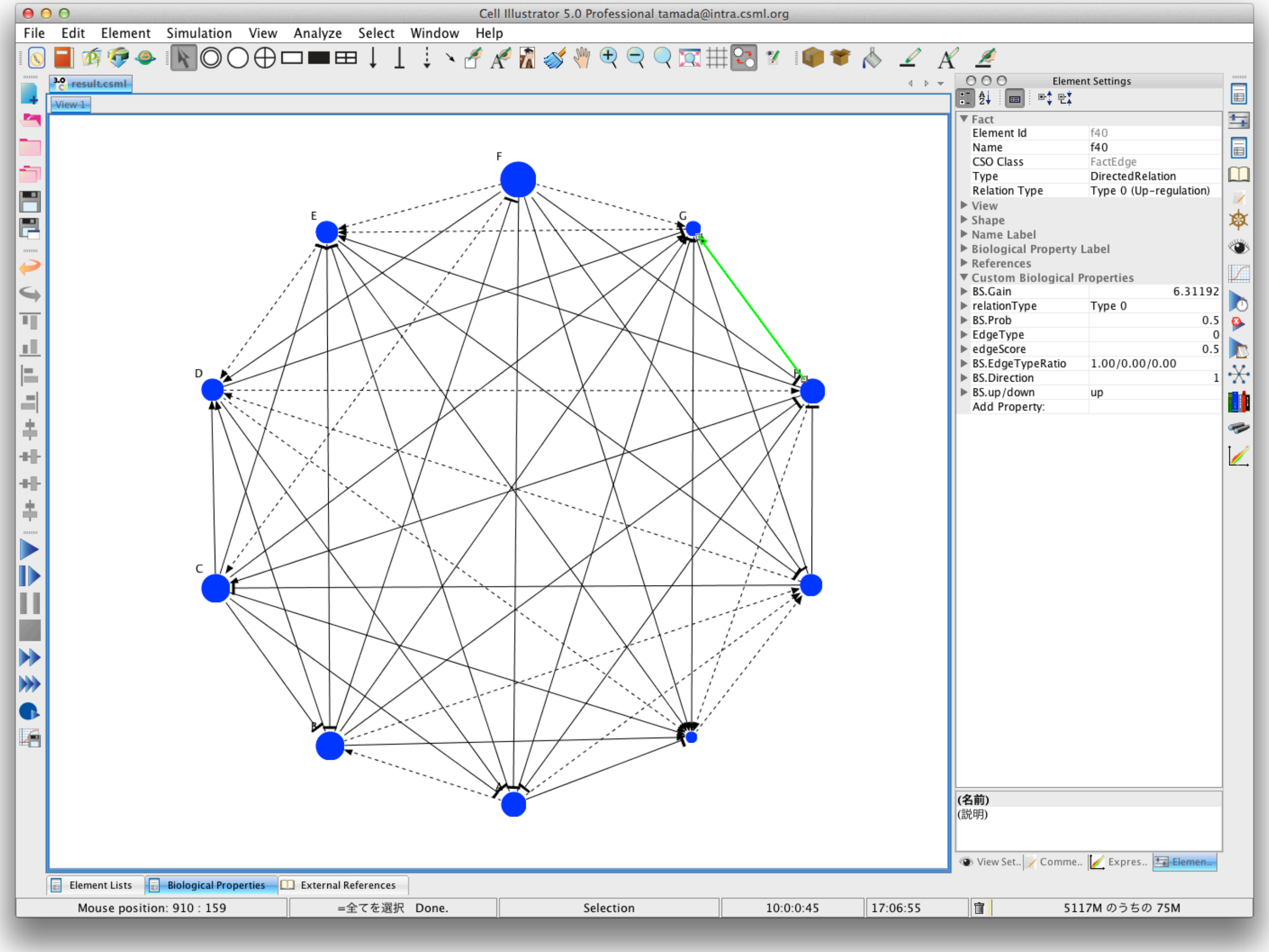
```
qsub [SGE options] /usr/local/bin/signbn-hc.sh --bin
   signproc --bs prefix=file_prefix [,other options] --output
   file=output_file,type=file_type
```

※ SGE の -t オプションは付けない

file_type は CSML か TXT

Cell Illustrator Online による解析

推定したネットワーク (CSML) は CIO で閲覧・編集・解析が可能



▼ Fact	
Element Id	f40
Name	f40
CSO Class	FactEdge
Type	DirectedRelation
Relation Type	Type 0 (Up-regulation)
▶ View	
▶ Shape	
▶ Name Label	
▶ Biological Property Label	
▶ References	
▼ Custom Biological Properties	
▶ BS.Gain	6.31192
▶ relationType	Type 0
▶ BS.Prob	0.5
▶ EdgeType	0
▶ edgeScore	0.5
▶ BS.EdgeTypeRatio	1.00/0.00/0.00
▶ BS.Direction	1
▶ BS.up/down	up
Add Property:	

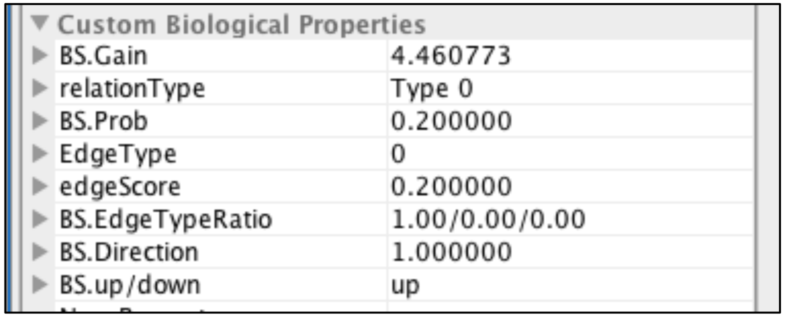
(名前)
(説明)

Mouse position: 910 : 159 | =全てを選択 Done. | Selection | 10:0:0:45 | 17:06:55 | 5117M のうちの 75M

Edge Properties

The following information is generated and assigned to each estimated edge. You can see them on CI Online.

`BS.Prob`	Bootstrap probability
`edgeScore`	Same as `BS.Prob`.
`BS.Gain`	Average of the edge gain
`BS.up/down`	One of “up”, “down”, or “unknown” that represents the estimated type of regulation.
`BS.edgeTypeRatio`	The ratio of up, down, and unknown regulation.
`BS.Direction`	Frequency (confidence) of the edge direction.



▼ Custom Biological Properties

▶ BS.Gain	4.460773
▶ relationType	Type 0
▶ BS.Prob	0.200000
▶ EdgeType	0
▶ edgeScore	0.200000
▶ BS.EdgeTypeRatio	1.00/0.00/0.00
▶ BS.Direction	1.000000
▶ BS.up/down	up

Edge properties shown in CI Online.

サンプルデータによる実習

（ベータ版）

サンプルファイル sample003.txt が ~tamada/sign/samples にあります.

(1) 出力用ディレクトリの準備と移動

```
mkdir ~/test
cd test
```

(2) SGEへのジョブの投入

赤字はベータ版でのみの指定

```
qsub -t 1-10 ~tamada/sign/signbn-hc.sh --dir ~tamada/sign --bs -o
   result ~tamada/sign/samples/sample003.txt
```

(3) SGEのジョブの確認

```
qstat
```

(4) 出力ファイルのまとめ

```
qsub ~tamada/sign/signbn-hc.sh --dir ~tamada/sign --bin signproc --bs
   prefix=result --output file=result.csml,type=csml
```

~/test に result.csml が作られます. ローカルPCに転送しCIOで開いてください.

Acknowledgments

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