Multiscale Bootstrap Analysis of Gene Networks Based on Graphical Gaussian Modeling

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Introduction

One of the purposes of microarray analysis is to estimate the relationships among genes. However, the estimation is often susceptible by statistical sampling error, and thus the result is obtained only by chance without reflecting the true hypothesis. Therefore, it is necessary to evaluate the reliability of hypothesis obtained as the result of analysis.

In this study, we present the program[1], which is written in R language, to assess the confidence of the gene network based on graphical Gaussian model by giving the *p*-value for the edges connecting genes. The main thrust of the program is to calculate the *p*-value of Approximately Unbiased (AU) test using the multiscale bootstrap resampling [4,5,6]. This method was developed recently to improve the accuracy of the bootstrap probability, and has been used widely in phylogenetic analysis.[2]

[1] We developed a program for the analysis as an add-on package for a statistical package R. It will be available at our website [8].
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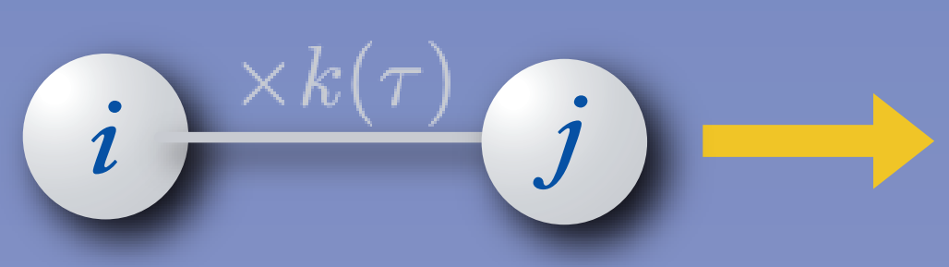
Bootstrap and Multiscale Bootstrap Edge Intensity

We measure the intensity of the edge by the bootstrap and multiscale bootstrap method. In the multiscale bootstrap method, we generate replicates $\boldsymbol{X}^*_{n'} = (\boldsymbol{x}^*_1, \dots, \boldsymbol{x}^*_{n'})$ for several n' values from the original gene expression data $\boldsymbol{X}_n = (\boldsymbol{x}_1, \dots, \boldsymbol{x}_n)$. In other words, we alter the number of arrays from n to n' in the bootstrap replication. We will take n' values with $n'/n =$ 0.5,0.6,0.7,0.8,0.9,1.0,1.1,1.2,1.3,1.4, in the example shown later. We call $\tau = \sqrt{n/n'}$ scale.

Bootstrap Algorithm with n' arrays

Step1: Generate the bootstrap replicate $\boldsymbol{X}^*_{n'}$.
Step2: Estimate the gene network from $\boldsymbol{X}^*_{n'}$.
Step3: Iterate Step1 and Step2 B times. Then we obtain B gene networks.
Step4: If the edges $gene_i \leftrightarrow gene_j$ exsist $k(\tau)$ times in the B networks, we define the bootstrap edge intensity between $gene_i$ and $gene_j$, $BP_{ij}(\tau)$, as $k(\tau)/B$.



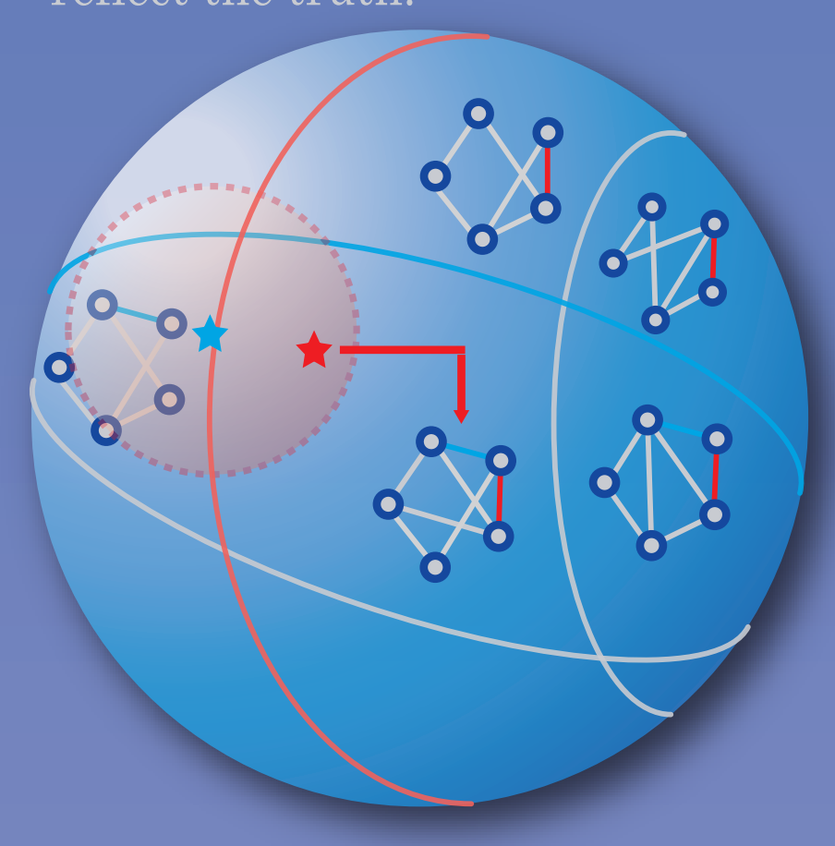
The bootstrap edge intensity

$$\boxed{BP_{ij}(\tau) = k(\tau)/B}$$

The data space divided by the resulting gene network

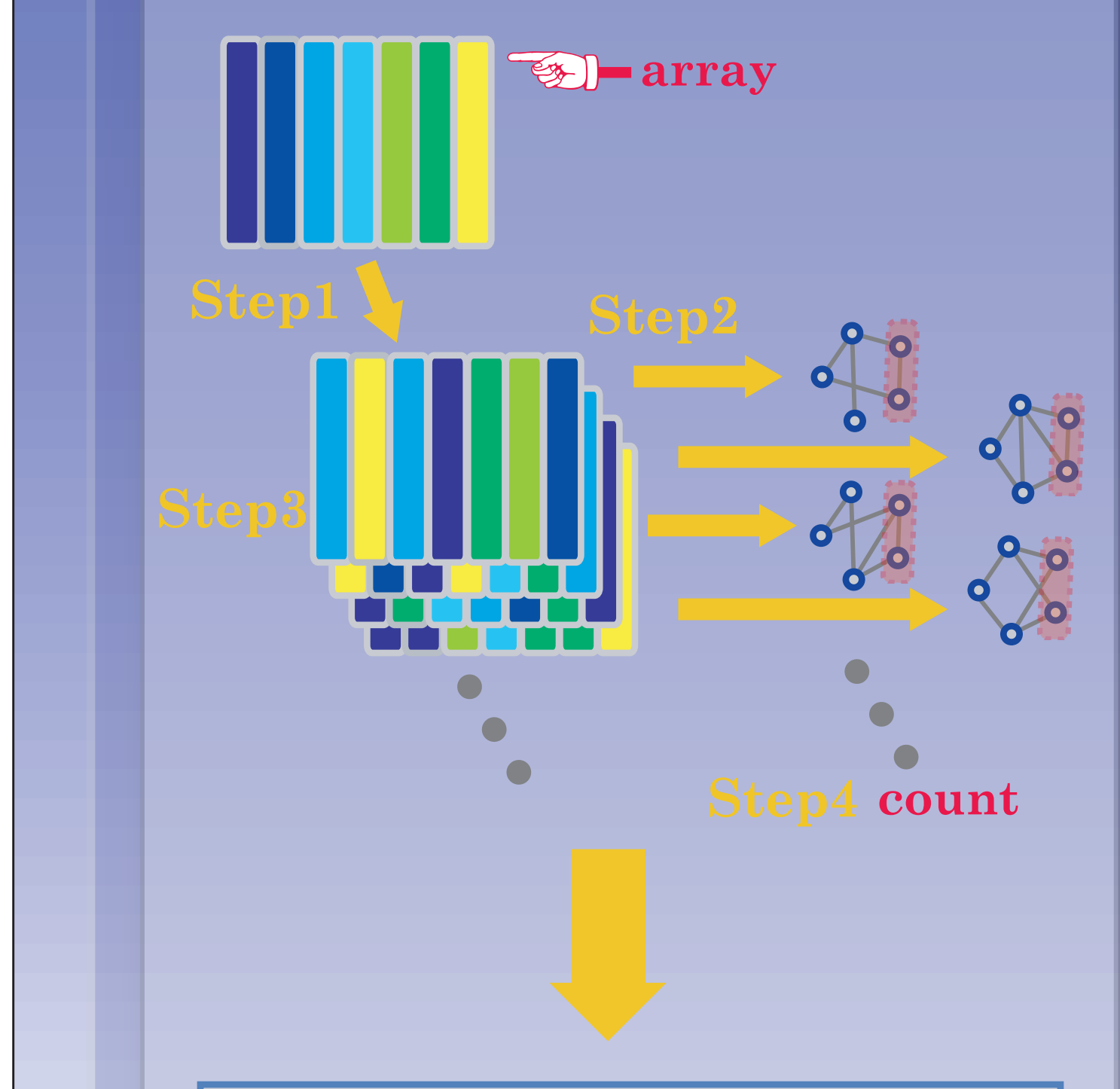
★ : sample data
★ : center of the probability distribution

→ The estimation is obtained because of random noise and does not correctly reflect the truth.



Bootstrap method

- We take $n = n'$.
- The bootstrap edge intensity can be written as $BP_{ij}(1)$.



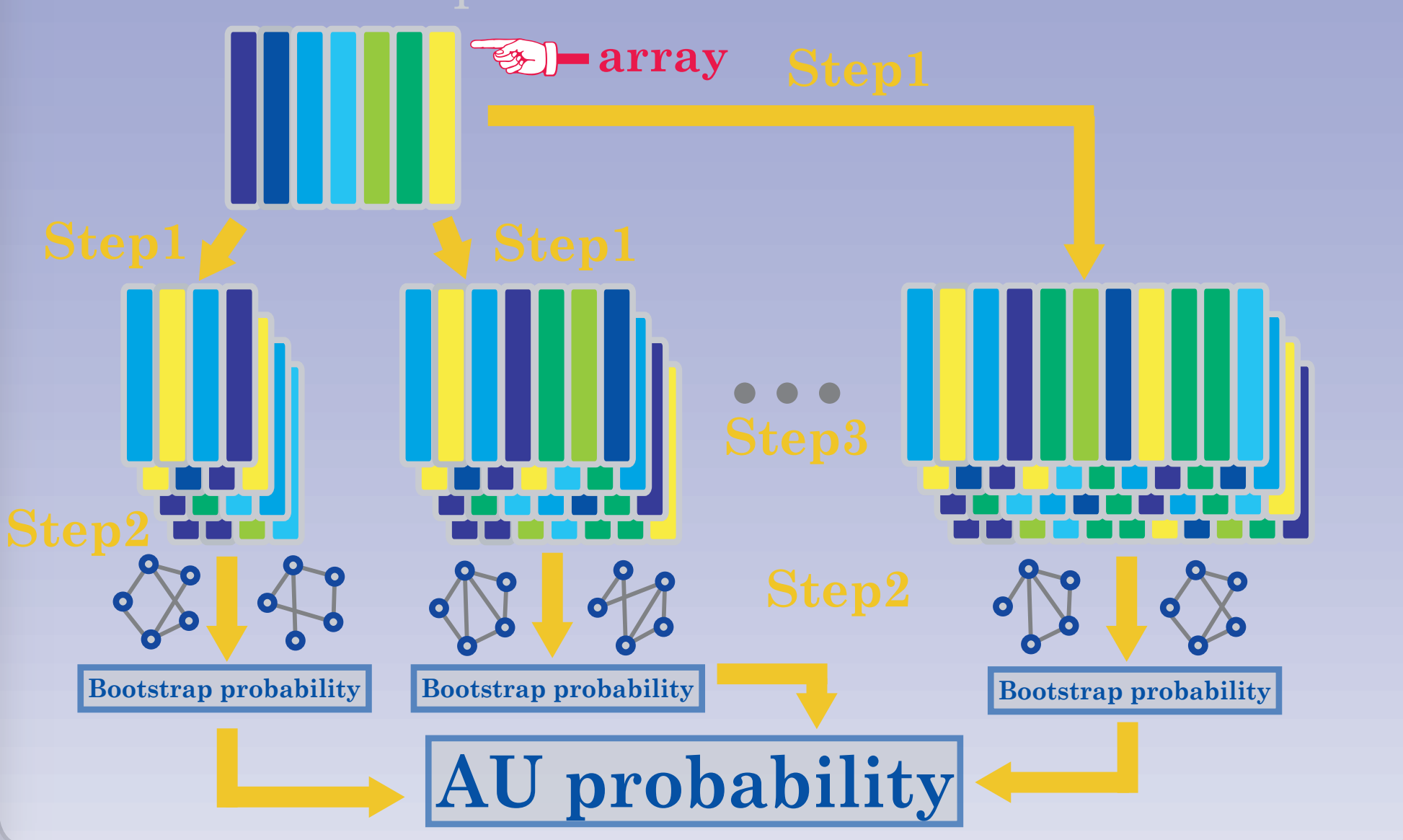
Bootstrap probability

Multiscale bootstrap method

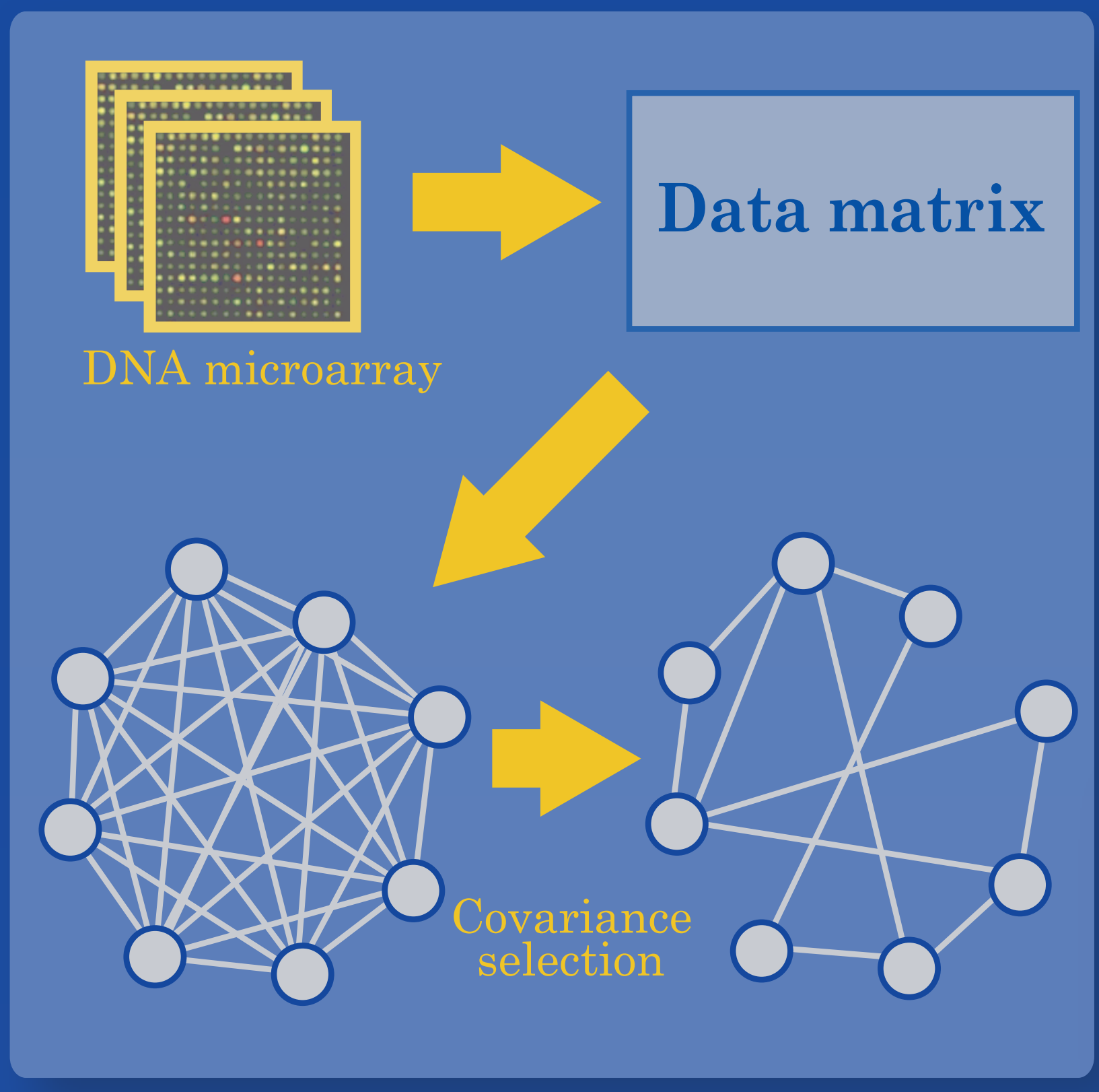
- We calculate $BP_{ij}(\tau)$ with several τ values by altering n'/n.

The very accurate edge intensity is expressed as $AU_{ij} = 1 - \Phi(d_{ij} - c_{ij})$.
d_{ij} : signed distance
c_{ij} : curvature
Φ : distribution function of standard normal distribution

- We estimated d_{ij} and c_{ij} by fitting the theoretical curve $BP_{ij}(\tau) = 1 - \Phi(d_{ij}/\tau + c_{ij}\tau)$ to the observed $BP_{ij}(\tau)$ values calculated by the multiscale bootstrap method.



Graphical Gaussian Model



Graphical Gaussian model assumes multivariate normal distribution for observed data and measure conditional independence relationships between two random variables based on partial correlation coefficient.

conditionally independent
→ not connected
otherwise
→ connected

A Numerical Example

- We applied the program to *S.cervisiae* gene expression data.
- We focused on 9 genes, which are involved or putatively involved in the heat shock response.
- We took B =10,000.

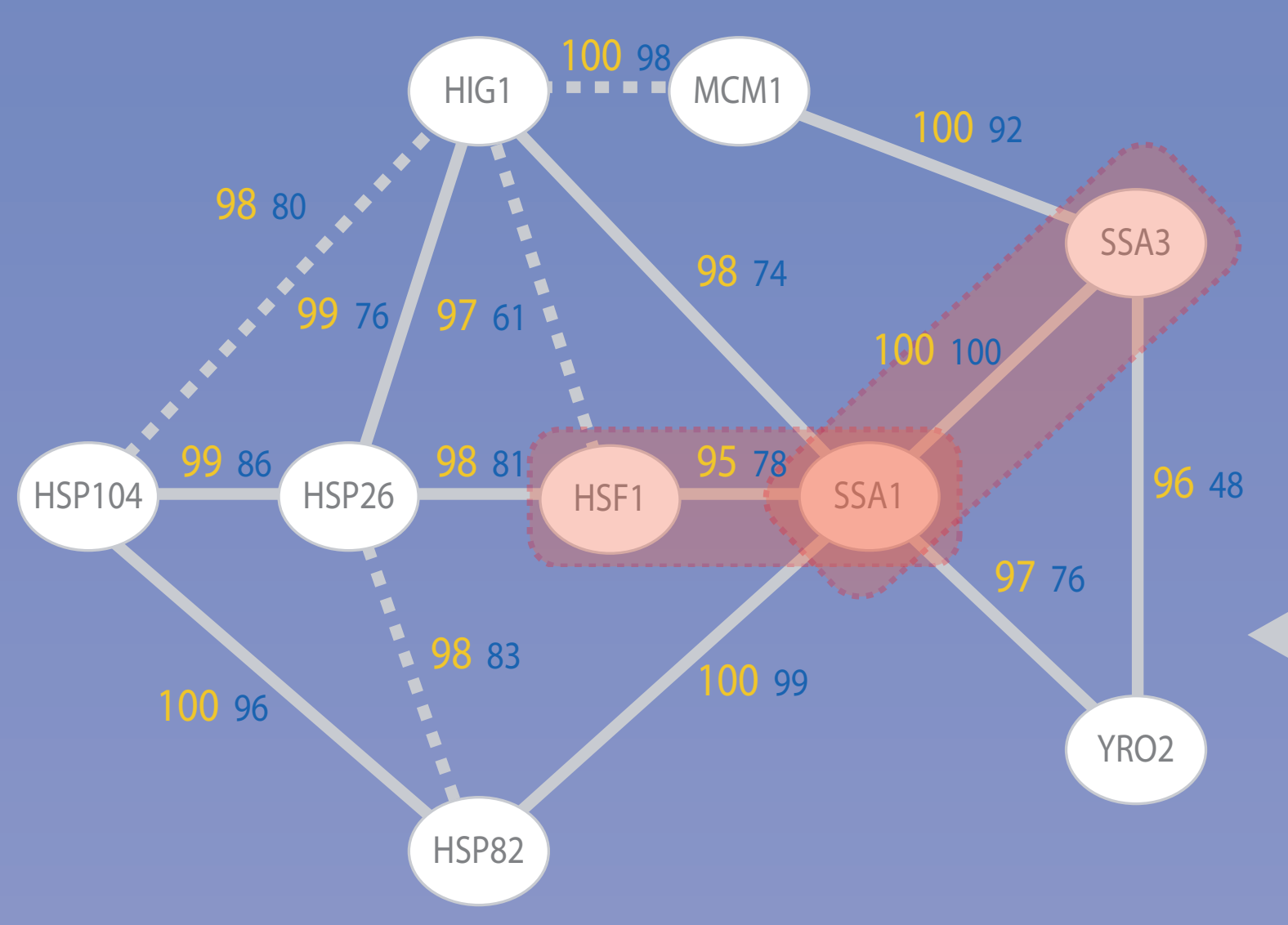
Matrix of the multiscale bootstrap edge intensities

$(AU_{ij}, 1 \leq j < i \leq 9)$

In our program, once you select the microarray data to analyze and give the number of replicates, all the multiscale bootstrap edge intensities in the network are calculated automatically.

HSP104	−								
HSF1	0.842	−							
HIG1	0.982	0.969	−						
MCM1	0.884	0.632	0.999	−					
SSA1	0.885	0.951	0.978	0.275	−				
SSA3	0.469	0.595	0.714	0.996	1.000	−			
HSP26	0.993	0.978	0.991	0.601	0.399	0.305	−		
HSP82	0.999	0.854	0.323	0.543	1.000	0.353	0.979	−	
YRO2	0.542	0.943	0.514	0.926	0.972	0.956	0.609	0.408	−
	HSP104	HSF1	HIG1	MCM1	SSA1	SSA3	HSP26	HSP82	YRO2

Estimated gene network



:AU probability (%)
:bootstrap probability (%)
:negative partial correlation
:positive partial correlation

◀ The estimated network captured the dependence of HSF1 and SSA1, and SSA1 and SSA3, which is reported in [2, 3].

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