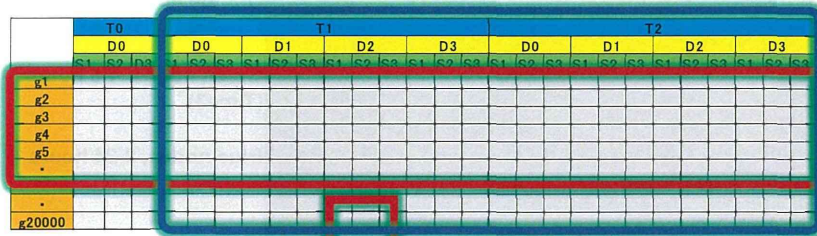


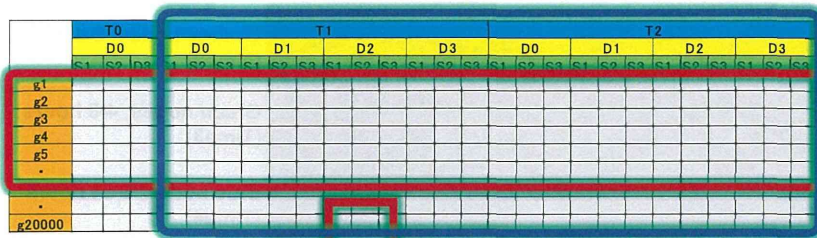
Inference of a network at 2 – 4 hours



94 genes started to change their expression value at 2 hours
 55 genes started to change their expression value at 4 hours.

The changes occurred from 2 hours to 4 hours.

Inference of a network at 2 – 4 hours



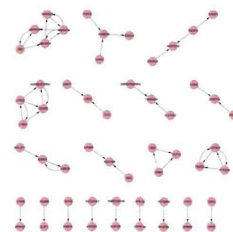
94 genes started to change their expression value at 2 hours
 55 genes started to change their expression value at 4 hours.

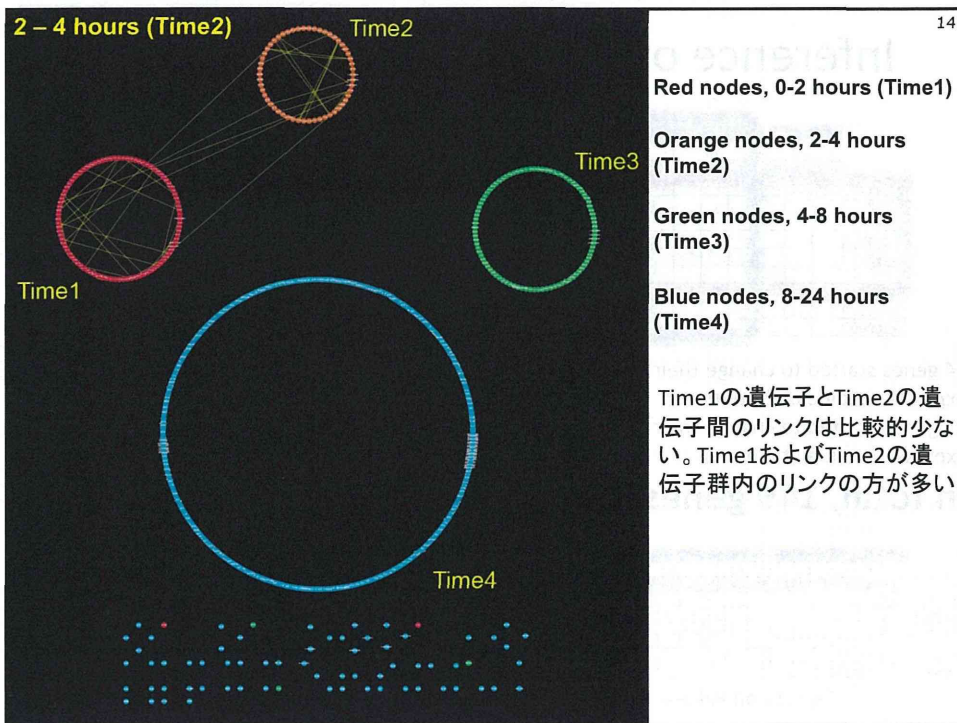
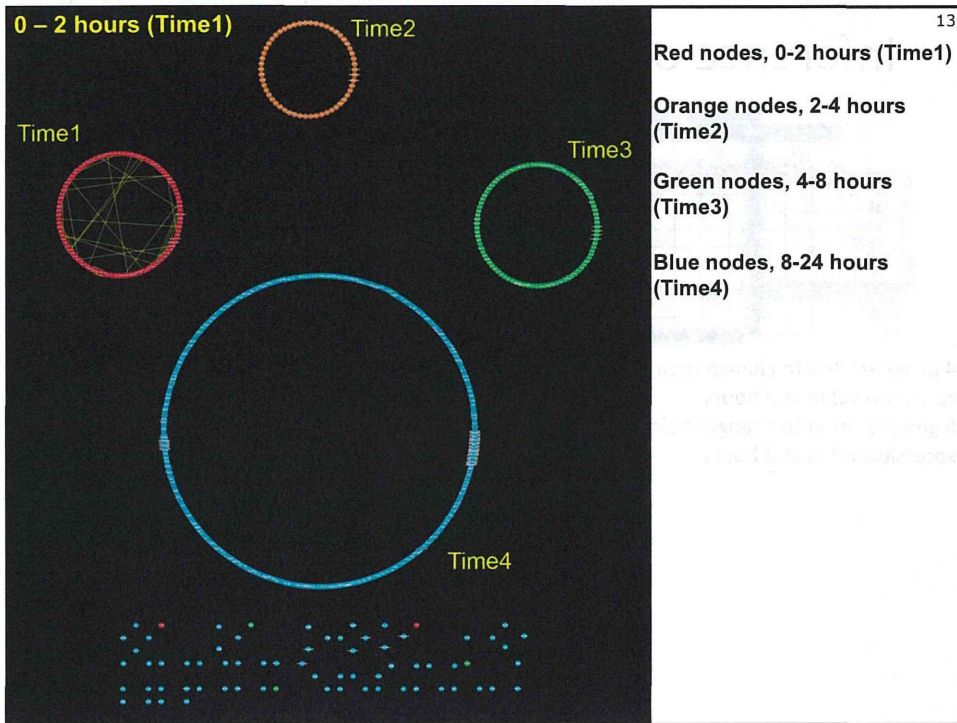
The changes occurred from 2 hours to 4 hours.

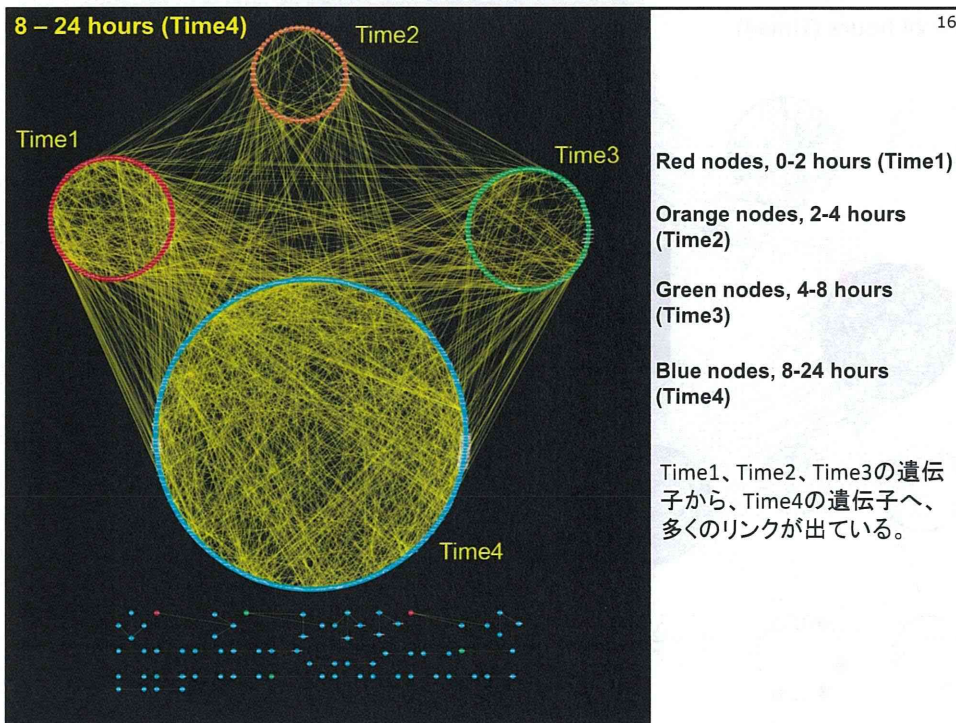
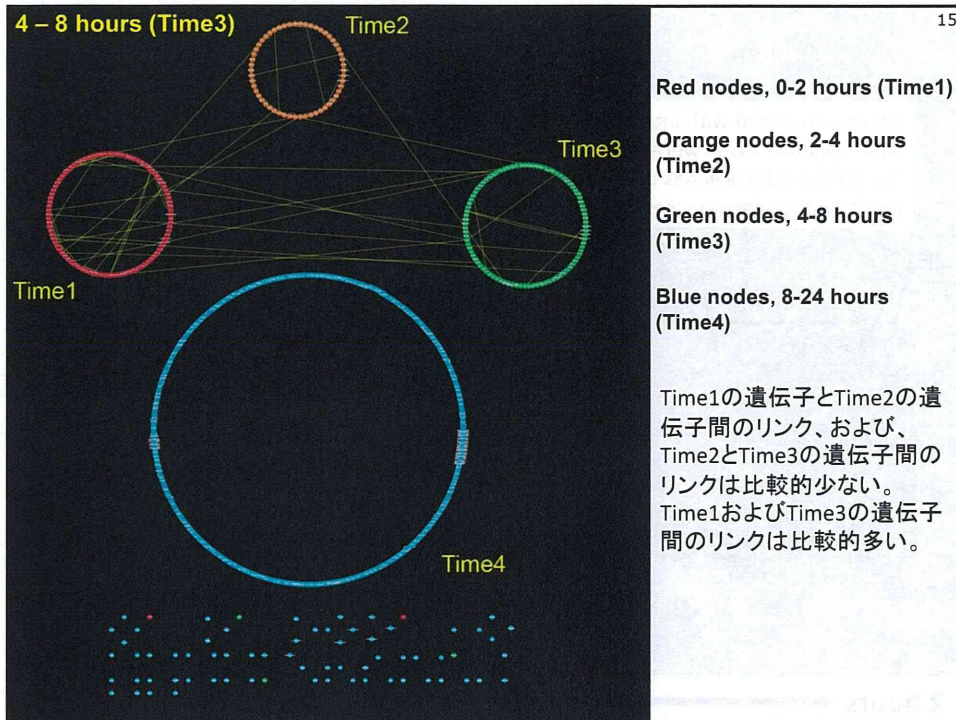
In total, 149 genes.

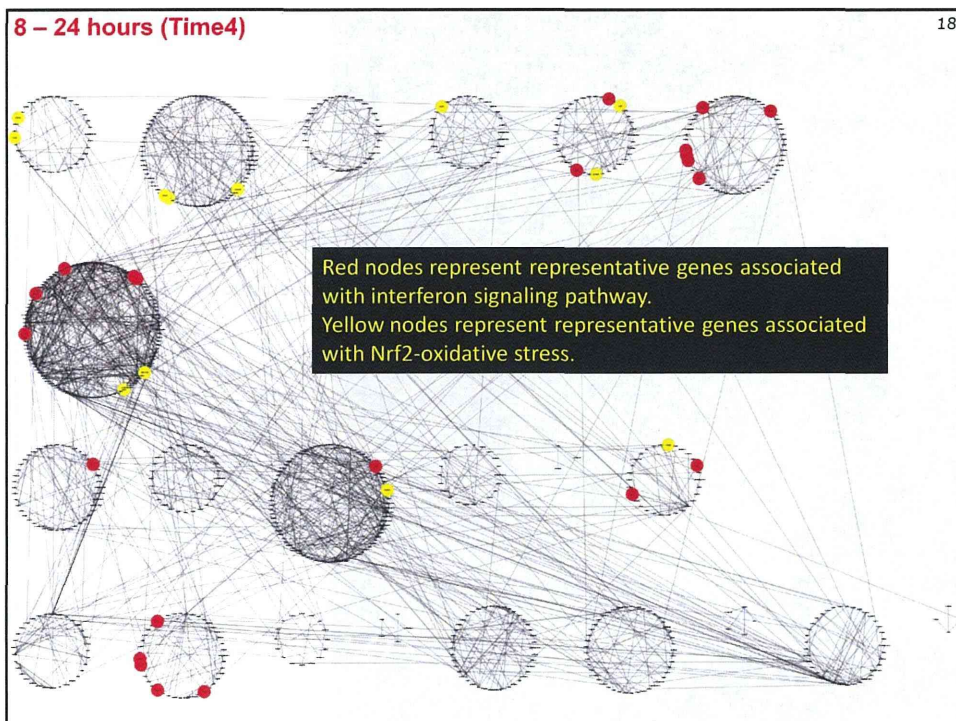
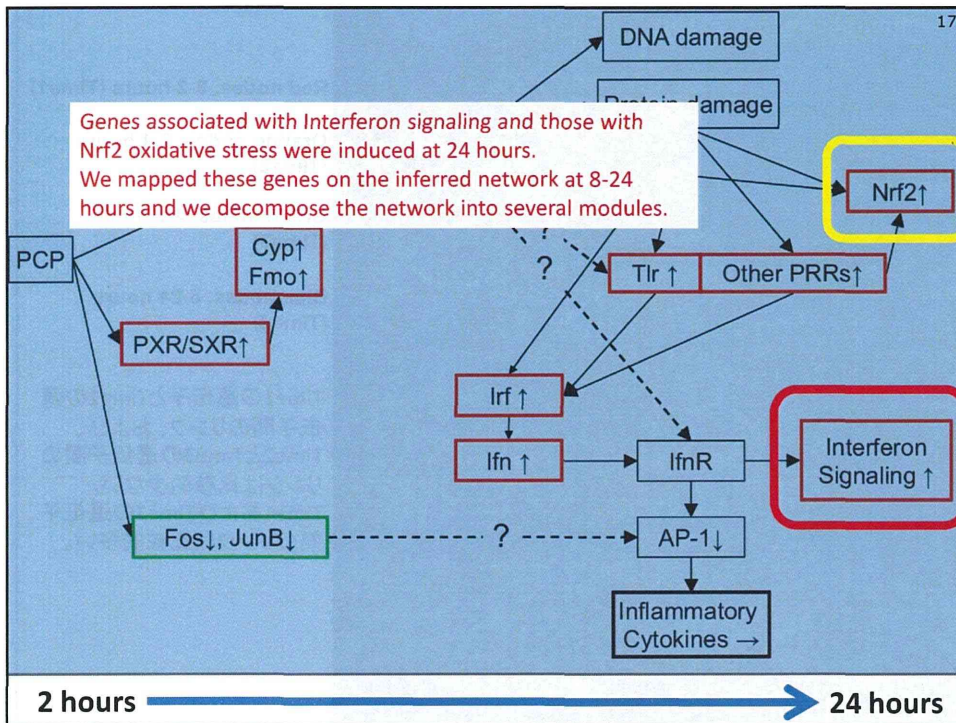


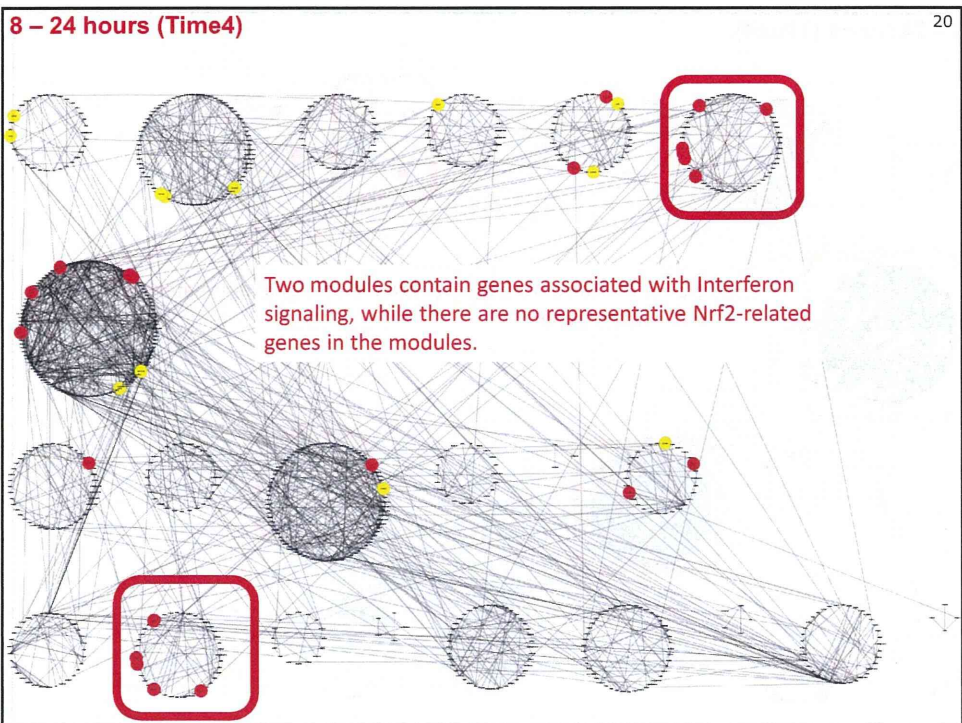
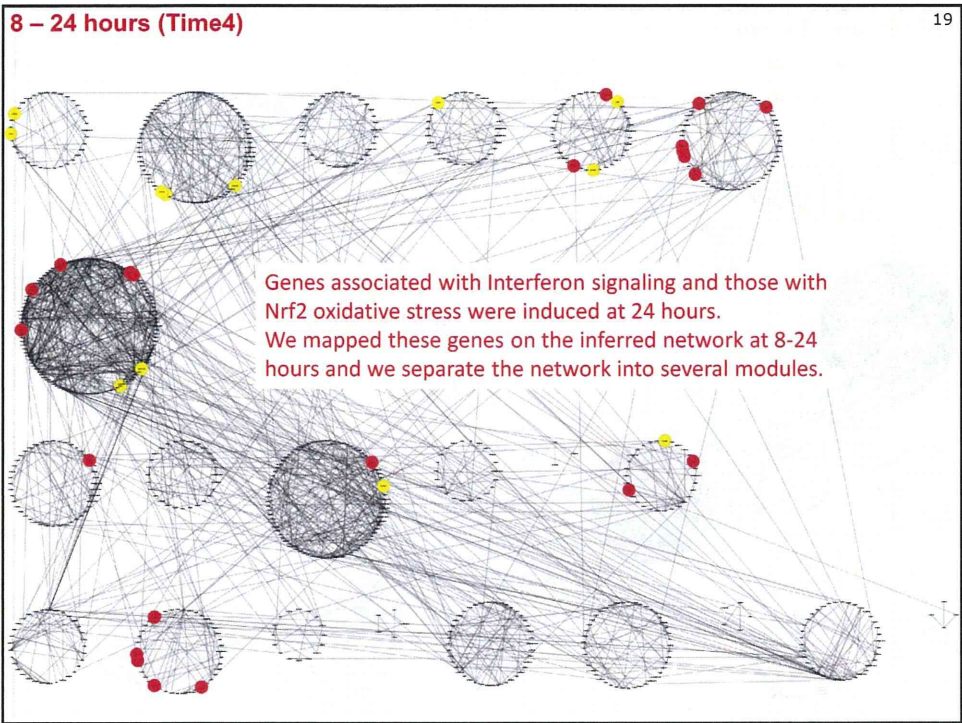
Expression values of 149 genes at 2 – 4 hours





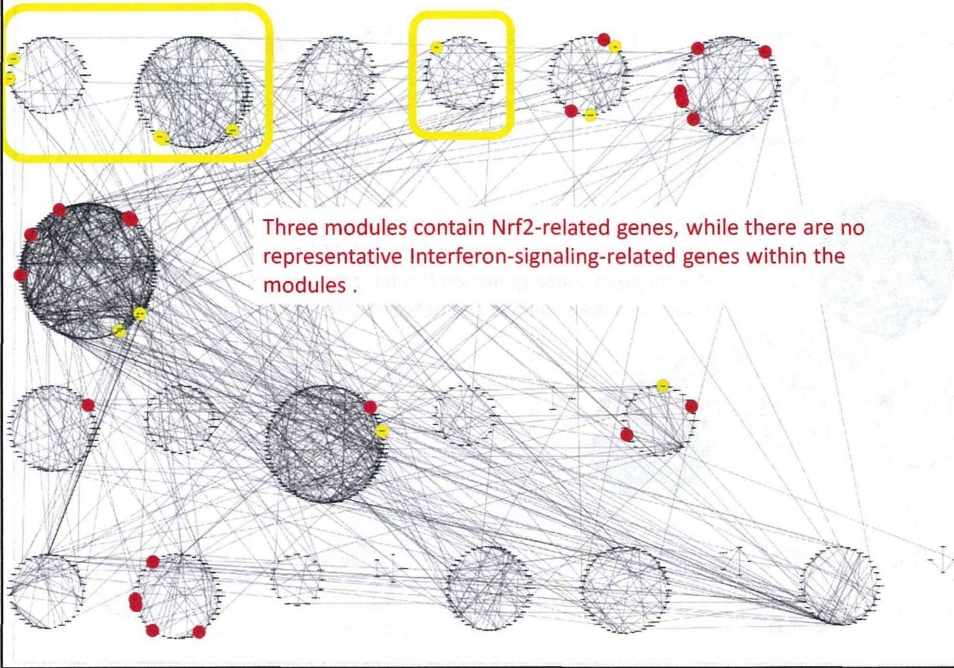






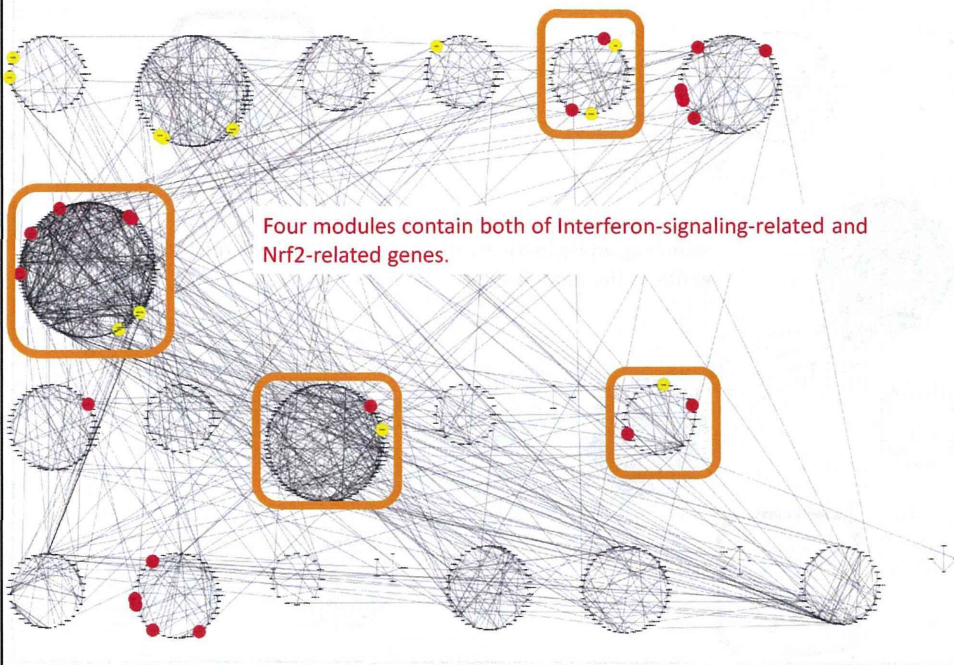
8 – 24 hours (Time4)

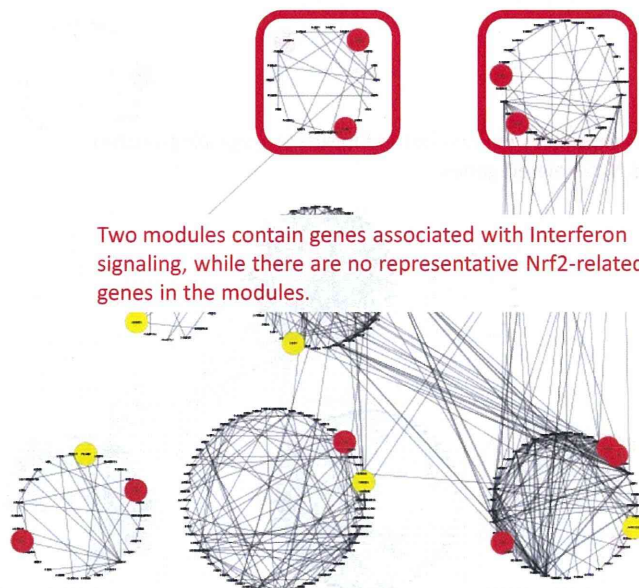
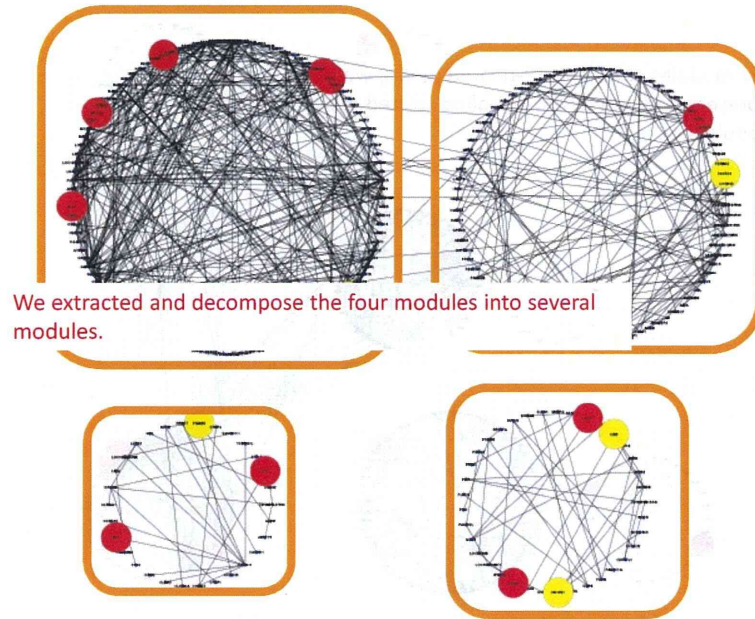
21



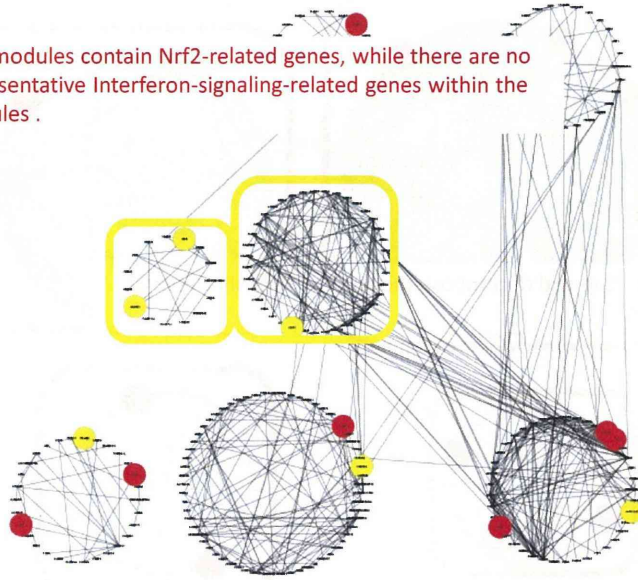
8 – 24 hours (Time4)

22

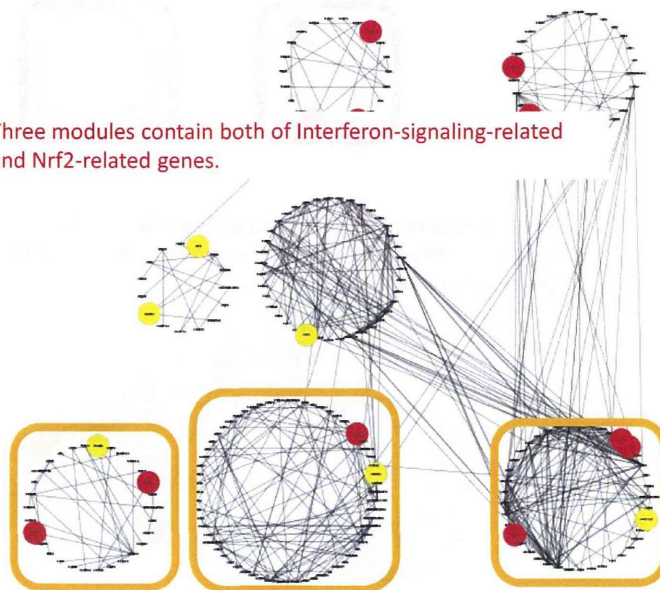




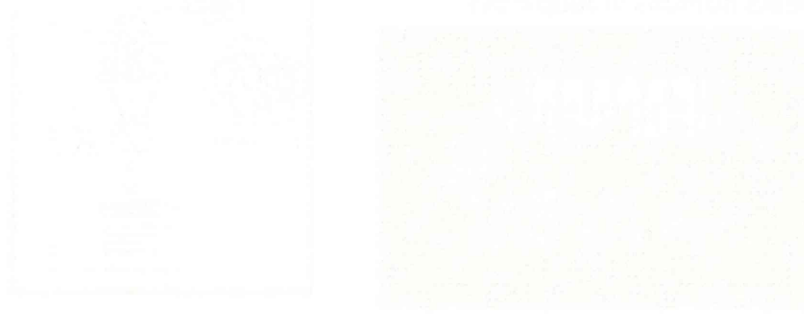
Two modules contain Nrf2-related genes, while there are no representative Interferon-signaling-related genes within the modules .



Three modules contain both of Interferon-signaling-related and Nrf2-related genes.



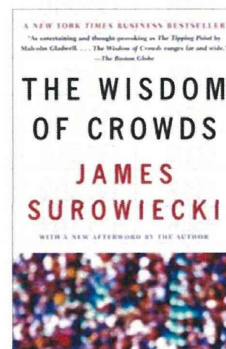
Development of network- inference algorithms



Harnessing Diversity

- Observation
 - Different algorithms have their strengths and weaknesses, assumptions and work well for specific data sets
- Can we harness this diversity to develop a network inference algorithm which gives better performance than the individuals?

**Wisdom of the Crowds in
biological network inference**

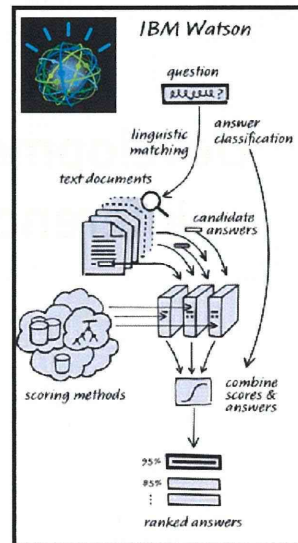


Harnessing Diversity

Jeopardy! is an American television quiz show featuring trivia in history, literature, and other topics.

The show has a unique answer-and-question format in which contestants are presented with clues in the form of answers, and must phrase their responses in question form

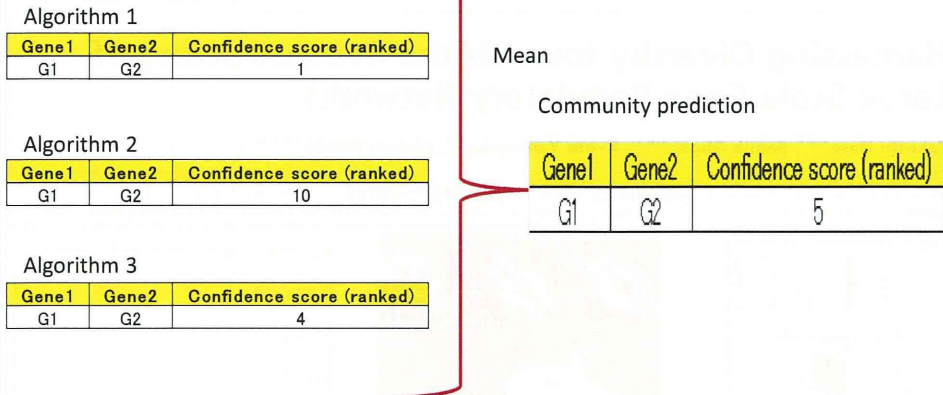
IBM's Watson supercomputer beats humans in Jeopardy!



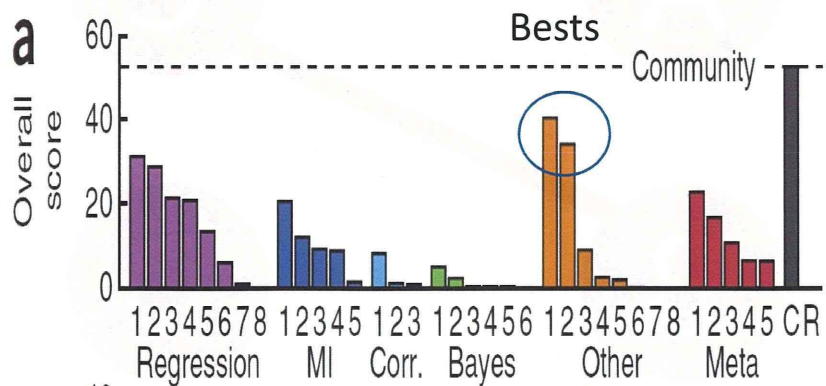
Confidence score from a single algorithm

Gene1	Gene2	Confidence score	Rank
G109	G1406	0.197688	10
G188	G938	0.193092	9
G49	G978	0.182752	8
G48	G1588	0.176977	7
G26	G741	0.173107	6
G48	G981	0.173	5
G95	G470	0.172894	4
G10	G1312	0.172802	3
G48	G1099	0.170831	2
G95	G1106	0.16546	1

Community prediction; integration of multiple algorithms



Community prediction; Overall score



Our community prediction method (Topknet method)

33

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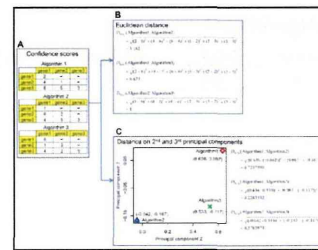
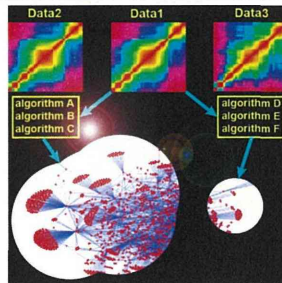
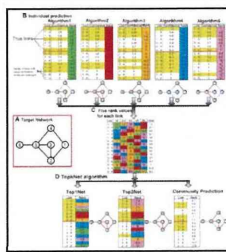
PLOS COMPUTATIONAL BIOLOGY

Harnessing Diversity towards the Reconstructing of Large Scale Gene Regulatory Networks

Takeshi Hase^{1,2*}, Samik Ghosh^{1,2,3*}, Ryota Yamanaka^{1,3}, Hiroaki Kitano^{1,2,4,5*}

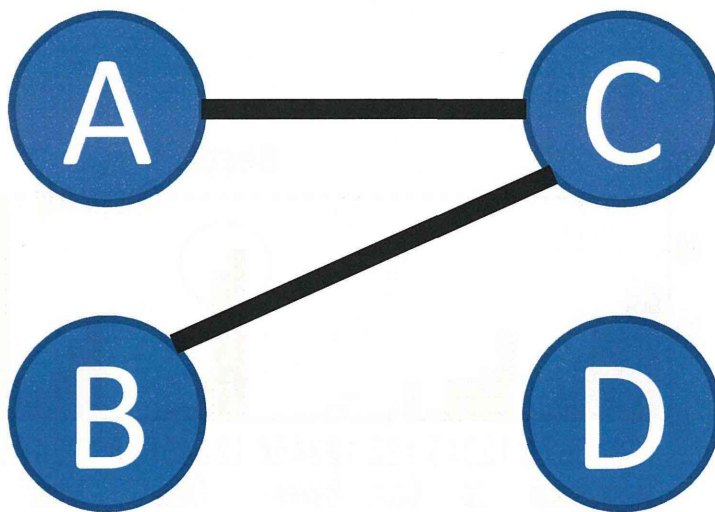
¹ The Systems Biology Institute, Shirokanedai, Atsugi-City, Kanagawa, Japan, ² Center for Integrative Medical Sciences, RIKEN, Suehiro-cho, Tsurumi-ku, Yokohama City, Kanagawa, Japan, ³ Center for Information Science, RIKEN, Wako, Saitama, Japan, ⁴ Sony Computer Science Laboratories, Inc., Higashigotanda, Shinagawa, Tokyo, Japan, ⁵ Okinawa Institute of Science and Technology, Onna, Onna-son, Kunigami, Okinawa, Japan

published on 21st November 2013

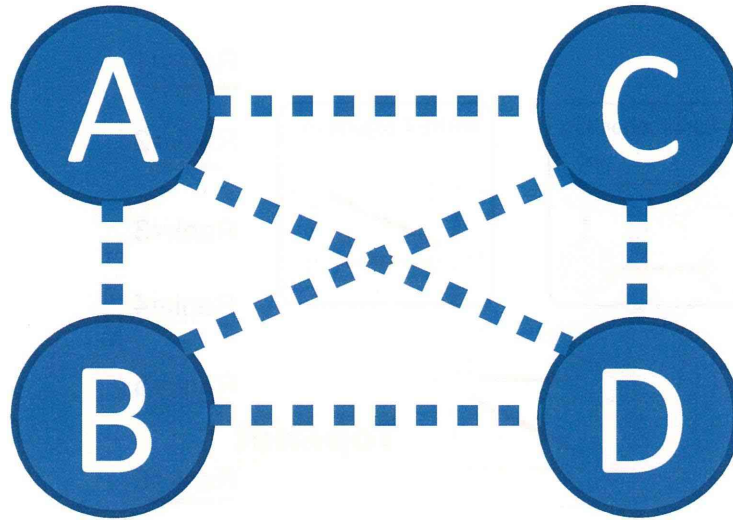


34

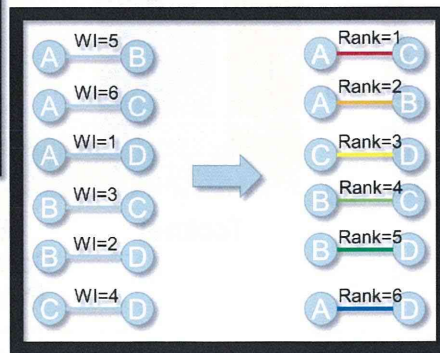
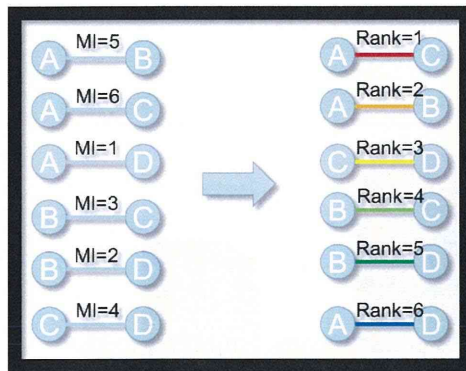
True Network



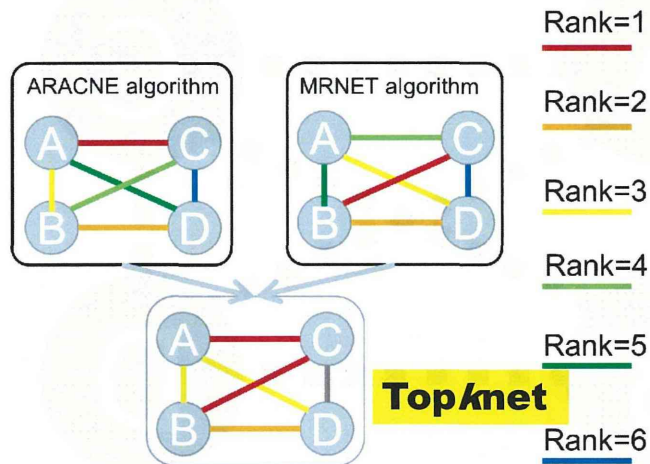
Many possibilities



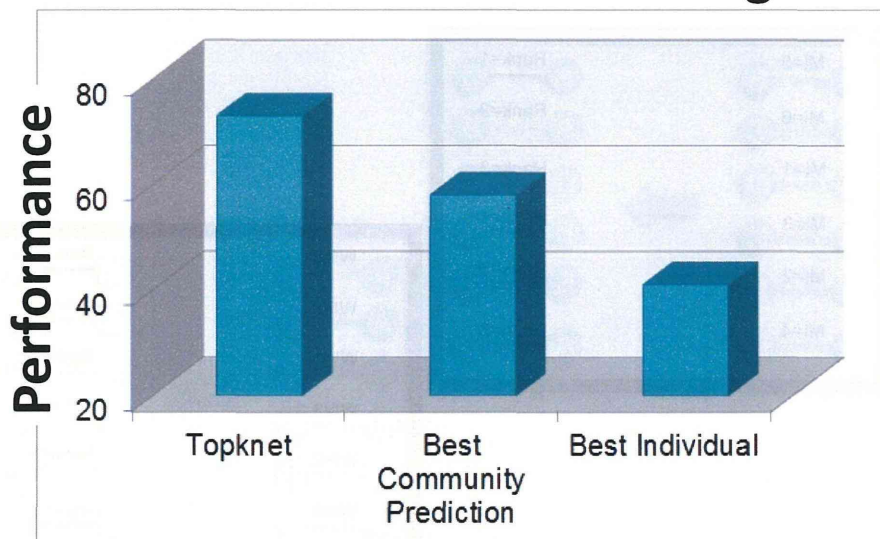
Each algorithm gives a version of the network



Wisdom of Crowds (Toknet)



Performance benchmarking



Topknet outperforms the best community prediction method and the best individual algorithm. Thus, network inferred by Topknet is the most reliable among networks by the other algorithms.

Topknet

- Our Topknet integrates 20 network-inference algorithms based on diverse statistical techniques (e.g., mutual-information, regression, bayesian, random forest, graphical gaussian model).
- Topknet will be one of key technologies to capture the effect of various drugs and chemical at network level.

Summary

- Ability to infer gene regulatory networks is a key problem in many biomedical research areas. We developed novel algorithms, “Topknet” that can infer reliable gene-regulatory networks from a large-scale gene expression dataset.
- Systems Toxicology endeavors to capture the effect of various drugs and chemical at network level.
- The Systems Toxicology project will provide one of the first applications of network-inference techniques to large scale toxicology databases with wide range of chemicals.
- The integration of Percellome data and associated gene-regulatory network by Topknet on the Garuda Platform will provide a unique global resource for systems toxicology studies.

Ⅲ. 研究成果の刊行に関する 一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Kanno J, Aisaki K, Igarashi K, Kitajima S, Matsuda N, Morita K, Tsuji M, Moriyama N, Furukawa Y, Otsuka M, Tachihara E, Nakatsu N, Kodama Y.	Oral administration of pentachlorophenol induces interferon signaling mRNAs in C57BL/6 male mouse liver.	J Toxicol Sci.	38 (4)	643 - 654	2013
Si Y, Inoue K, Igarashi K, Kanno J, Imai Y.	Autoimmune regulator, Aire, is a novel regulator of chondrocyte differentiation.	Biochem Biophys Res Commun.	437 (4)	579 - 584	2013
Fujimoto N, Takagi A, Kanno J.	Neonatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin increases the mRNA expression of prostatic proteins in C57BL mice.	J Toxicol Sci.	38 (2)	279 - 283	2013
Hase T, Ghosh S, Yamanaka R, Kitano H.	Harnessing Diversity towards the Reconstructing of Large Scale Gene Regulatory Networks.	PLOS Computational Biology	9 (11)		2013
Matsuoka Y, Matsumae H, Katoh M, Einfeld AJ, Neumann G, Hase T, Ghosh S, Shoemaker JE, Tiago Lopes, Watanabe T, Watanabe S, Fukuyama S, Kitano H, Kawaoka Y.	A comprehensive map of the influenza A virus replication cycle.	BMC Systems Biology	7, 97		2013

Yamashita F, Sasa Y, Yoshida S, Hisaka A, Asai Y, Kitano H, Hashida M, Suzuki H.	Modeling of Rifampicin-Induced CYP3A4 Activation Dynamics for the Prediction of Clinical Drug-Drug Interactions from In Vitro Data.	PLOS ONE	8,9		2013
Fujita K, Ostaszewski M, Matsuoka Y, Ghosh S, Glaab E, Trefois C, Crespo I, Perumal TM, Jurkowski W, Antony PMA, Diederich N, Buttini M, Kodama A, Satagopam VP, Eifes S, Del Sol A, Schneider R, Kitano H, Balling R.	Integrating Pathways of Parkinson's Disease in a Molecular Interaction Map.	Molecular Neurobiology	49	88 - 102	2014
Naito T, Yatsunami A, Kaji N, Ando T, Sato, K, Moriya H, Kitano H, Yasui T, Tokeshi M, Baba Y.	Parallel Real-Time PCR on a Chip for Genetic Tug-of-War (gTOW) Method.	Analytical Sciences	29 (3)	367 - 371	2013
Makanae K, Kintaka R, Makino T, Kitano H, Moriya H.	Identification of dosage-sensitive genes in <i>Saccharomyces cerevisiae</i> using the genetic tug-of-war method.	Genome Research	23	300 - 311	2013
Schaefer MH, Lopes T, Mah N, Shoemaker JE, Matsuoka Y, Fontaine JF, Louis-Jeune C, Einfeld AJ, Neumann G, Perez-Iratxeta C, Kawaoka Y, Kitano H, Andrade-Navarro MA.	Adding Protein Context to the Human Protein-Protein Interaction Network to Reveal Meaningful Interactions.	PLOS Computational Biology	9,1		2013
Polouliakh N	Reprogramming resistant genes: in-depth comparison of gene expressions among iPS, ES and somatic cells.	Frontier of Physiology	4 (7)		2013

IV. 研究成果の刊行物・別刷

Original Article

Oral administration of pentachlorophenol induces interferon signaling mRNAs in C57BL/6 male mouse liver

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Koichi Morita¹, Masaki Tsuji¹, Noriko Moriyama¹, Yusuke Furukawa¹, Maki Otsuka¹,
Erika Tachihara¹, Noriyuki Nakatsu² and Yukio Kodama¹

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(Received June 17, 2013; Accepted June 25, 2013)

ABSTRACT — Pentachlorophenol (PCP) was monitored for transcriptome responses in adult mouse liver at 2, 4, 8 and 24 hr after a single oral administration at four dose levels, 0, 10, 30 and 100 mg/kg. The expression data obtained using Affymetrix GeneChip MOE430 2.0 were absolutized by the Percellome method and expressed as three dimensional (3D) surface graphs with axes of time, dose and copy numbers of mRNA per cell. We developed the programs RSort, for comprehensive screening of the 3D surface data and PercellomeExploror for cross-referencing and confirmed the significant responses by visual inspection. In the first 8 hr, approximately 100 probe sets (PSs) related to PXR/SXR and Cyp2a4 and other metabolic enzymes were induced whereas Fos and JunB were suppressed. At 24 hr, about 1,200 PSs were strongly induced. We cross-referenced the Percellome database consisting of 111 chemicals on the liver transcriptome and found that about half of the PSs belonged to the metabolic pathways including Nrf2-mediated oxidative stress response networks shared with some of the 111 chemicals. The other half of the induced genes were interferon signaling network genes (ISG) and their induction was unique to PCP. Toll like receptors and other pattern recognition receptors, interferon regulatory factors and interferon alpha itself were included but inflammatory cytokines were not induced. In summary, these data indicated that functional symptoms of PCP treatment, such as hyperthermia and profuse sweating might be mediated by the ISG rather than the previously documented mitochondrial uncoupling mechanism. PCP might become a hint for developing low molecular weight orally available interferon mimetic drugs following imiquimod and RO4948191 as agonists of toll-like receptor and interferon receptor.

Key words: Pentachlorophenol, Mouse, Liver, Interferon signaling genes, Percellome toxicogenomics

INTRODUCTION

The Percellome Toxicogenomics Project is designed to identify dynamic and extensive networks of genes whose time- and dose-dependent patterns of expression in response to a chemical allows its toxic effects to be predicted. For this project, we developed a standardization method for microarrays and quantitative PCR that produces copy number of mRNAs per one cell (designated as “Percellome method”) (Kanno *et al.*, 2006). This method allowed us to directly and quantitatively compare gene expression data among samples, studies, organs and even species using four arithmetic operations. One hundred

and eleven chemicals (as of June 2013, Supplementary Table 1), most of which are known for their toxicity, were examined using the standard protocol of the project.

Pentachlorophenol (PCP) was examined in adult male C57BL/6 mouse liver. This compound has been used for multiple purposes such as herbicide, insecticide, fungicide, disinfectant, and other preservative purposes, moreover, its metabolism and toxicity, including carcinogenicity have been well studied. PCP is known to induce morphological changes in liver, kidney, hematopoietic, respiratory, immune and neural systems together with irritation of exposed sites. Hepatocarcinogenicity was demonstrated in rodents; the postulated mechanism involves

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