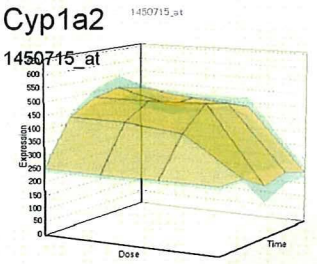
3-MC 30mg/kg

Cyp1a2

anti-sense

Cyp1a2



cytochrome P450, family 1, subfamily a, polypeptide 2

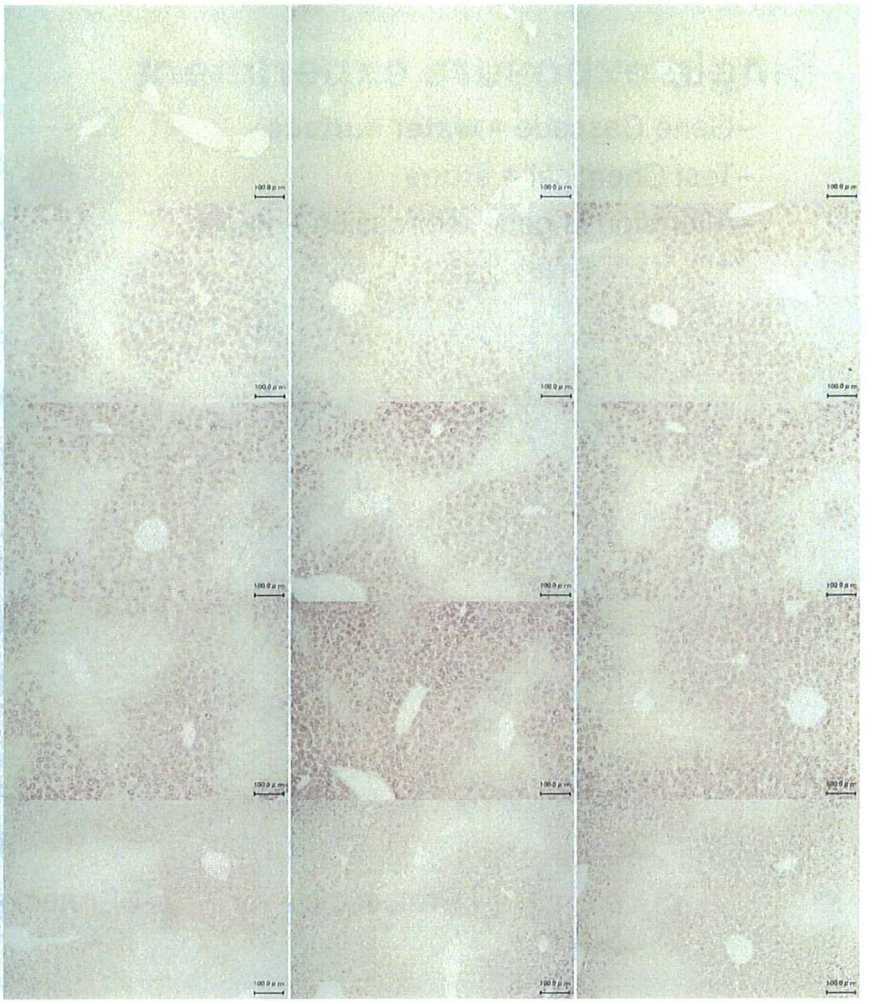
0hr

2hr

4hr

8hr

24hr



Strategy for data analysis

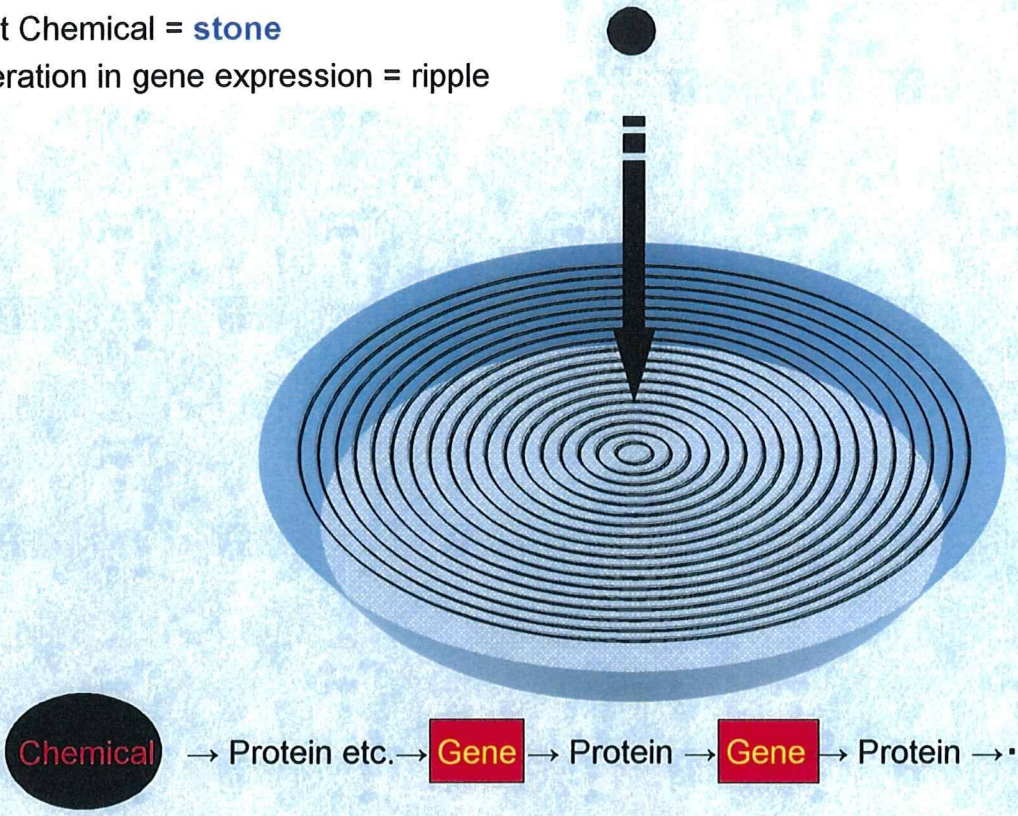
46

Single exposure experiment

–Gene Cascade = **water surface**

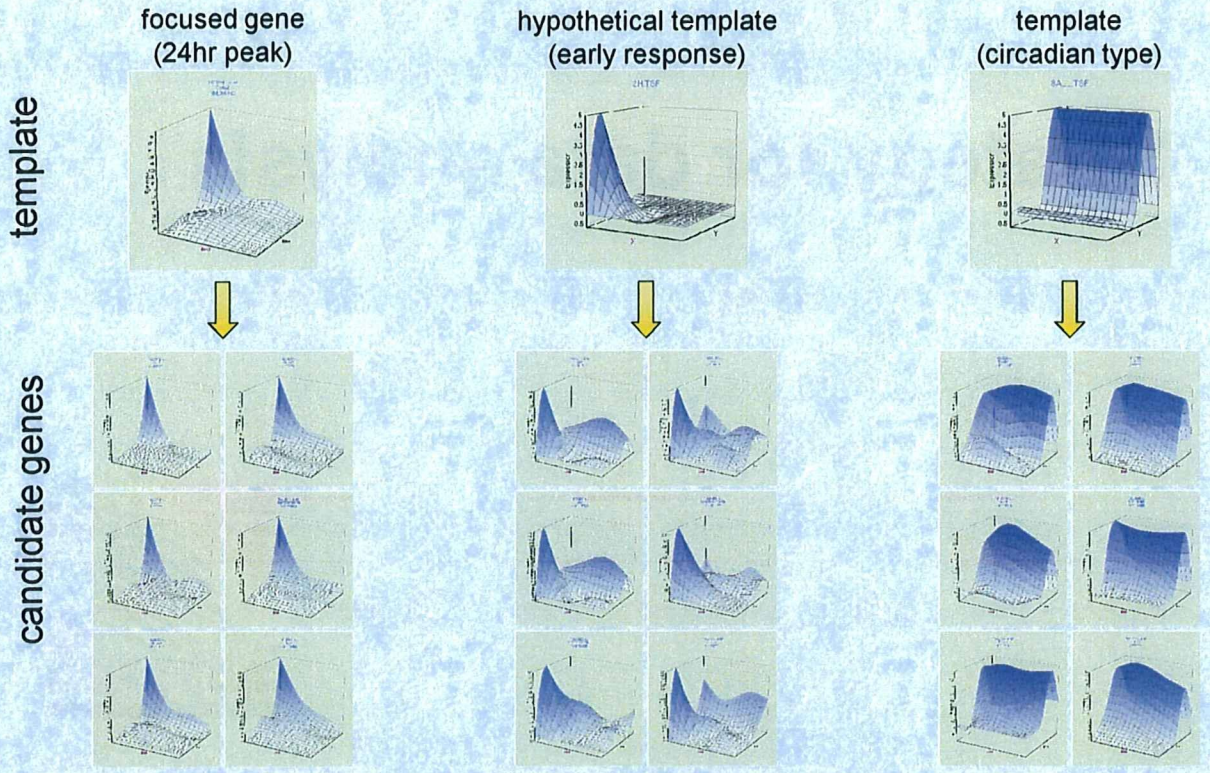
–Test Chemical = **stone**

–Alteration in gene expression = ripple

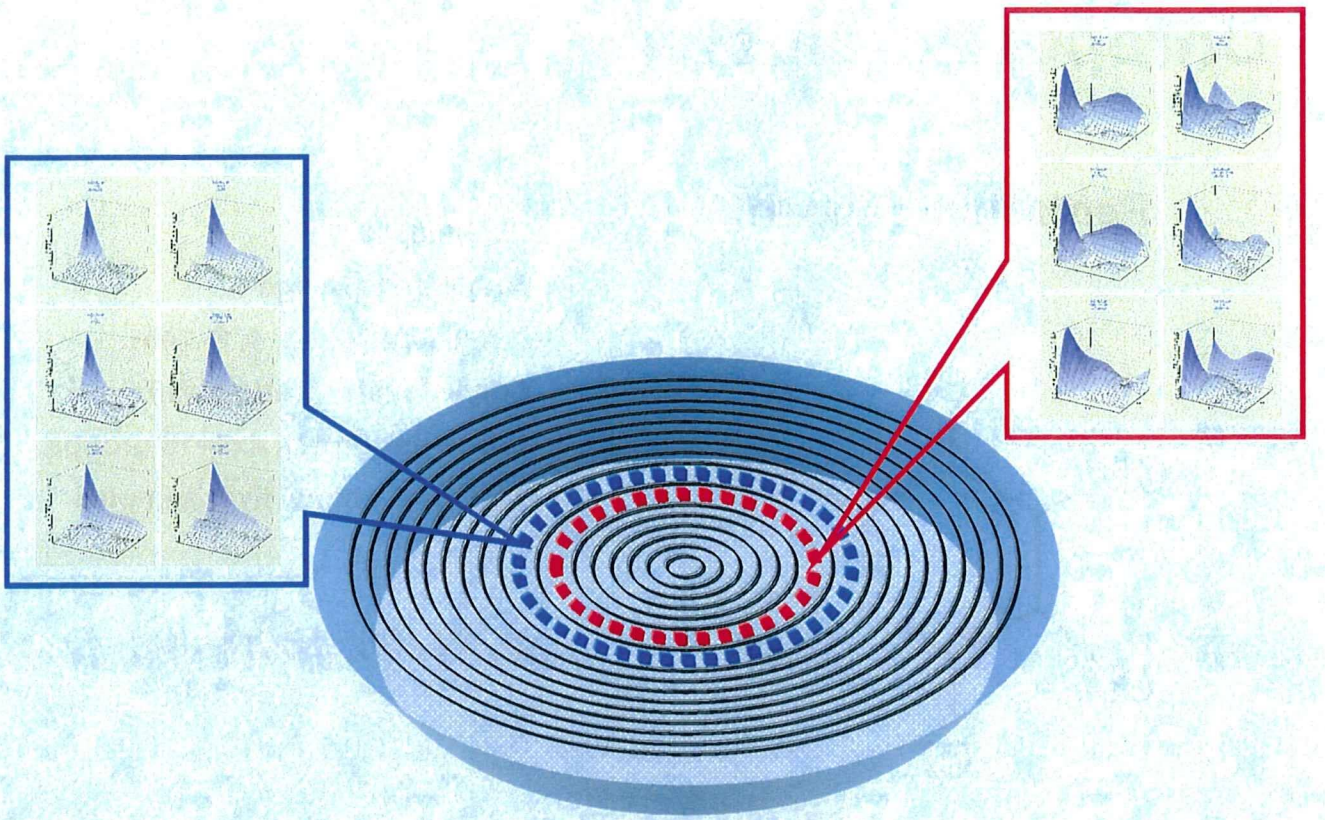


47

'Millefeuille' analysis system ~ Candidate Gene Extraction



48



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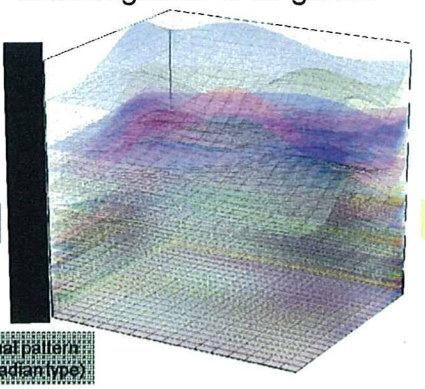
'Millefeuille(MF)' analysis system:

isoborograms of all genes



expression pattern-based automatic candidate gene extraction

unsupervised clustering

(Mass Distributed Clustering*)

- extracting unique expression-patterned genes
- sort 50,000 genes into 1,000 clusters

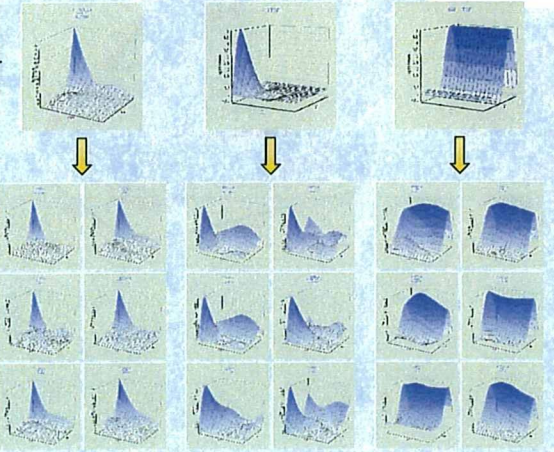
*collaboration with NTT COMWARE and Teradata (NCR)

focused gene (24hr peak)

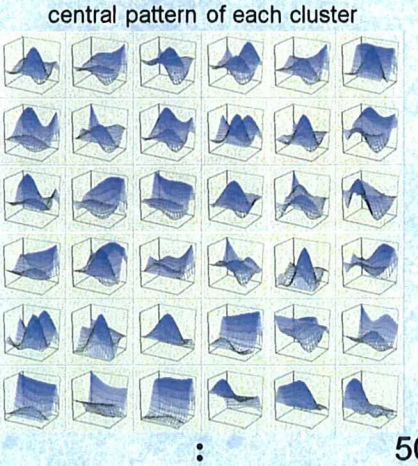
virtual pattern (early response)

virtual pattern (circadian type)

template



central pattern of each cluster



S. Matsumoto, et al.

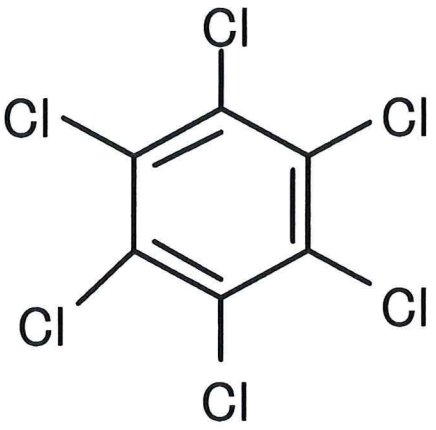
Mass Distributed Clustering: A New Algorithm for Repeated Measurements in Gene Expression Data.

Genome Informatics (2005), 16, 183-194

50

Unreported cluster

Pentachlorophenol (PCP)



–C57BL/6, 12W ♂
–Single oral gavage
–4 time points 2, 4, 8, 24hr
–4 dose levels (ratio = √10)
–3 mice per group, 4x4=16 groups,
–total of 48 animal liver samples

0, 10, 30, 100mg/kg

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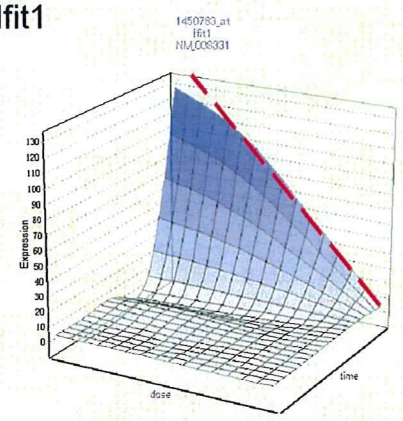
Dose-dependent increase at 24hr by PCP

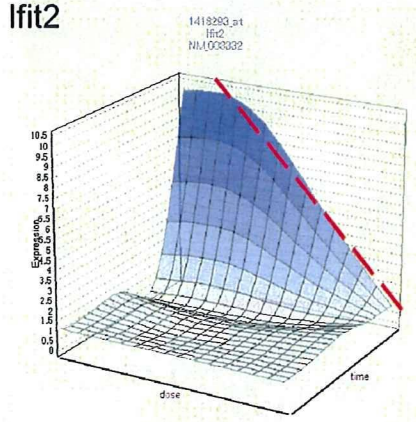
<< IFIT family gene >>

IFIT (interferon-induced protein with tetratricopeptide repeats)

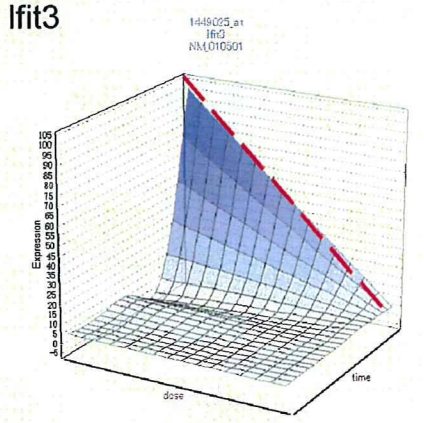
Ifit1



Ifit2



Ifit3



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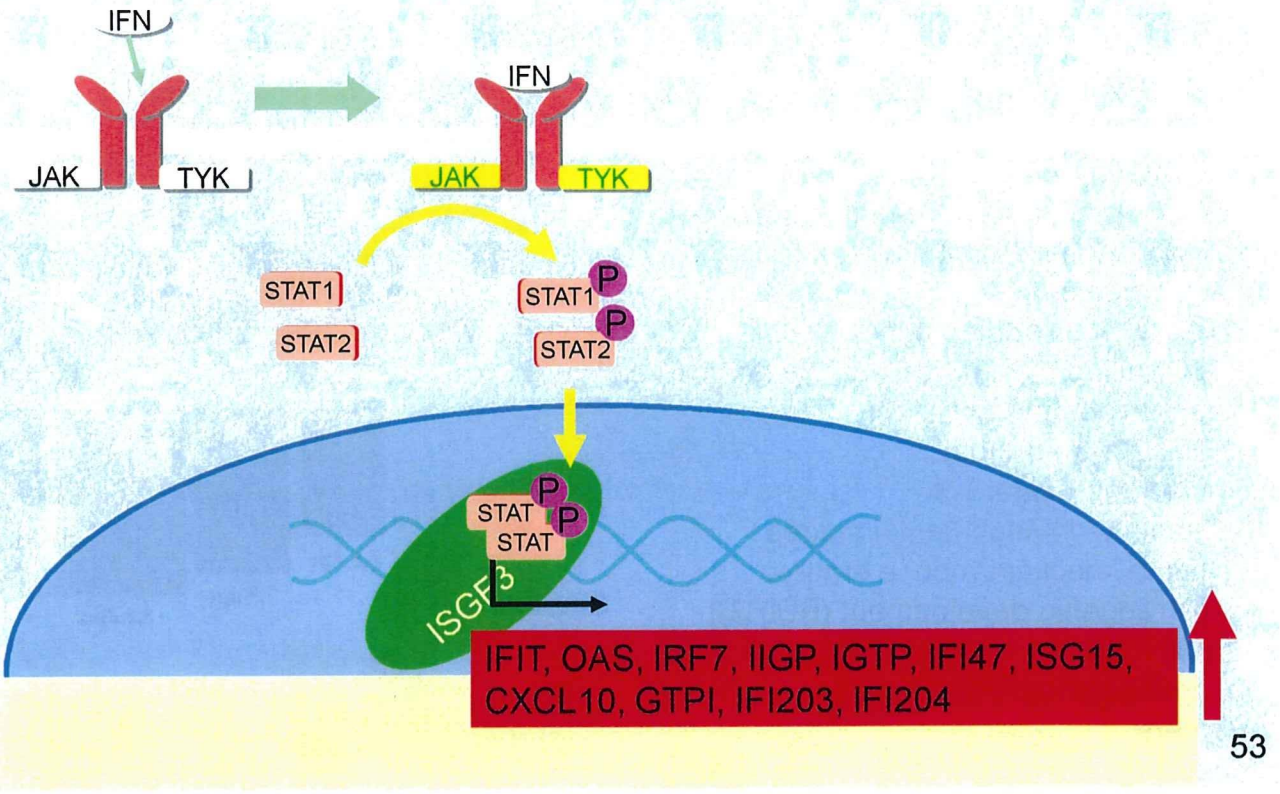
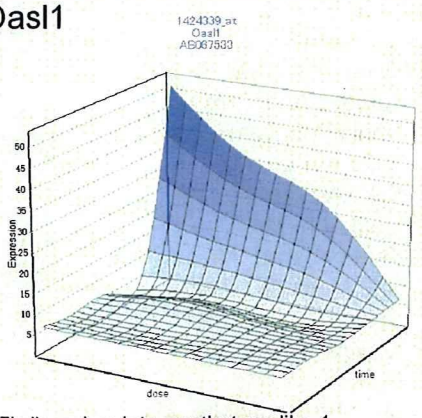
Interferon pathway



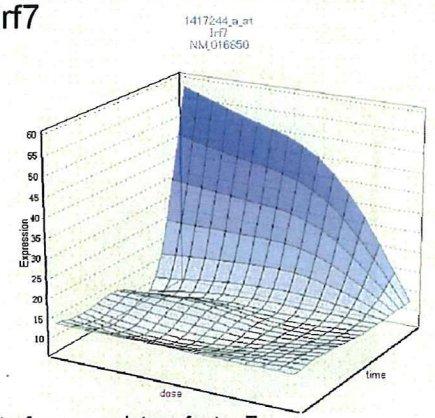
Other IFN-induced genes by PCP, including IFN signaling components

Oasl1



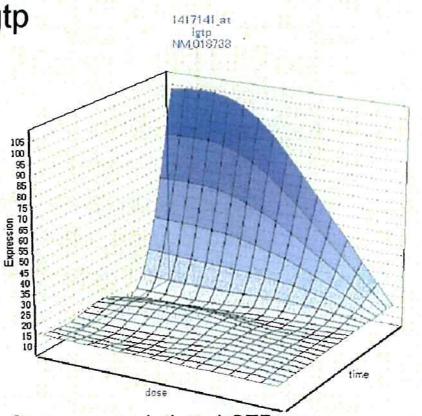
2'-5' oligoadenylate synthetase-like 1

Irf7



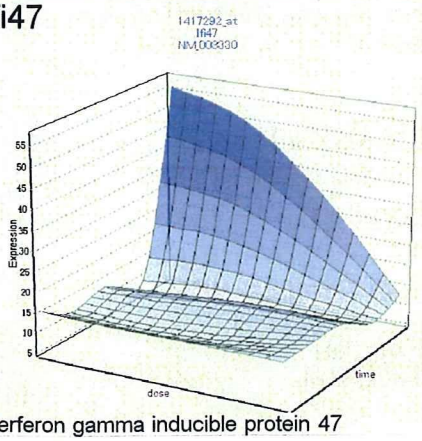
interferon regulatory factor 7

Igtp



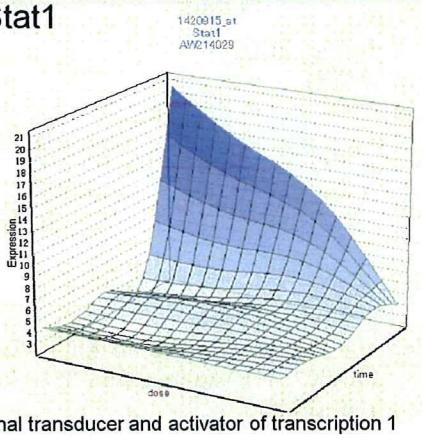
interferon gamma induced GTPase

Ifi47



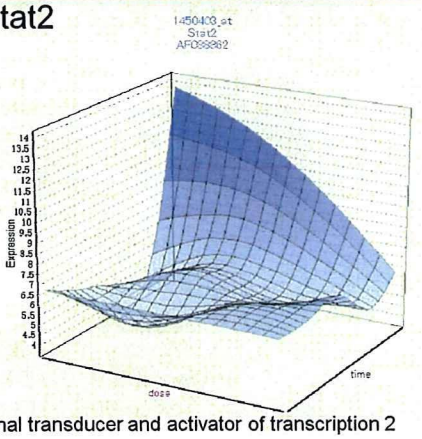
interferon gamma inducible protein 47

Stat1



signal transducer and activator of transcription 1

Stat2

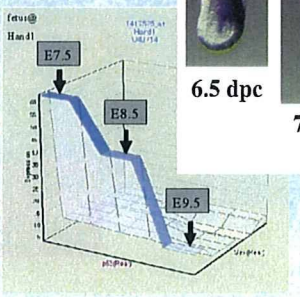


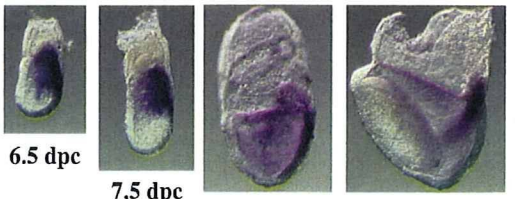
signal transducer and activator of transcription 2

Percellome Project (mouse)

@Div. Cellular and Molecular Toxicology/ BSRC/ NIHS

- TTG1: 2003~ (Adult mouse)
  - –single exposure, oral. liver 100 chem
  - –gene knockout mouse
- TTG2: 2006~ (Adult mouse)
  - –repeated exposure, oral
  - –multi-organ
  - –high-through put *in situ* hybridization
- **TTG3: 2009~** (Adult mouse)
  - –Informatics
- ITG: 2004~ (Adult mouse)
  - –inhalation (Lung & Liver), low dose level
  - –2hr exposure, 6hr, 22hr,
- FTG: 2003~ (Fetus)
  - –Developmental TG
  - –Various developmental stages
  - –Gene knockout mouse embryo
  - –[Phylogenic development (PDTG)]
- Food TG:
- Combine effect TG:
- Others





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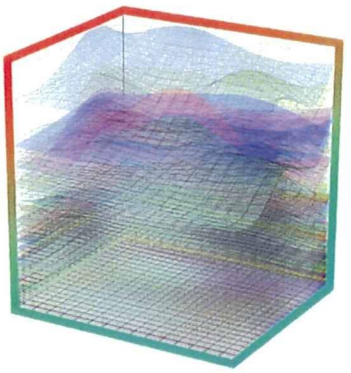
Development of Percellome (2001~)

Jun Kanno, MD, PhD
Katsuhide Igarashi, PhD
Ken-ichi Aisaki, MD, PhD
Atsushi Ono, PhD
Tomoko Ando, Ms
Noriko Moriyama, Ms
Yuko Kondo, Ms
Yuko Nakamura, Ms
Maki Abe, Ms



Percellome (Millefeuille) (2003~)

Ken-ichi Aisaki, MD, PhD
Katsuhide Igarashi, PhD
Noriyuki Nakatsu, PhD
Yukio Kodama, DVM
Tomoko Ando, Ms
Noriko Moriyama, Ms
Yuko Kondo, Ms
Yuko Nakamura, Ms
Maki Abe, Ms
Kenta Yoshiki, Mr
Nae Matsuda, DVM
Chiyuri Aoyagi, Ms
Kouichi Morita, Mr
Ayako Imai, Ms
Shinobu Watanabe, Ms
Yukio Ogawa, DVM (Inhalation)
Satoshi Kitajima DVM, PhD (Fetus)
Kentaro Tanemura DVM, PhD

NIHS TGP (with 17 Pharm) startup group (~summer 2002)

Akihiko Hirose	Risk Assess/ BSRC/ NIHS
Takayoshi Suzuki	Mutagen/ BSRC/ NIHS
Makoto Shibutani	Path/ BSRC/ NIHS
Katsuhide Igarashi	Tox/BSRC/NIHS
Atsushi Ono	Tox/BSRC/NIHS
Ken-ichi Aisaki	Tox/BSRC/NIHS
Jun Kanno	Tox/BSRC/NIHS

Millefeuille Softwares

Ken-ichi Aisaki, MD, PhD

IT collaboration

NTT COMWARE
with Teradata, NCR

Grants

Ministry of Health, Labor, and Welfare (MHLW) Grant-in-Aid H21-kagaku-ippan-001, H18-kagaku-ippan-001, H17-kagaku-003, H15-kagaku-002, H14-Toxico-001, H13-seikatsu-012, & MOE

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Fetus Percellome Toxicogenomics

57

AIM

Establishing the evaluation system concerning the developmental toxicity of high accuracy

↓

Fetus Percellome Toxicogenomics case study on a model teratogen

58

Background 1

Necessity of the evaluation system concerning the developmental toxicity of high accuracy

1) **Species difference**
   ex) Thalidomide
2) **Detection sensitivity**
   ex1) Spontaneous occurrence of embryotoxicity in the control group
   ex2) Difference of sensitivity among the embryos of the same litter
   ex3) Abnormality which tends to be overlooked
   [Functional disorder, Immune disorder, Changes in tumor incidence, Becoming a short life, etc

→ **Even if the embryotoxicity is not observed in the laboratory animal, it is necessary to foresee the embryotoxicity in the human accurately.**

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Background 2

Feature of embryo in view of the gene-expression

Unlike adult, in the case of embryo,

the expressions of the genes related to development change violently as time passes

→ **A specific signal cascade can be drawn**

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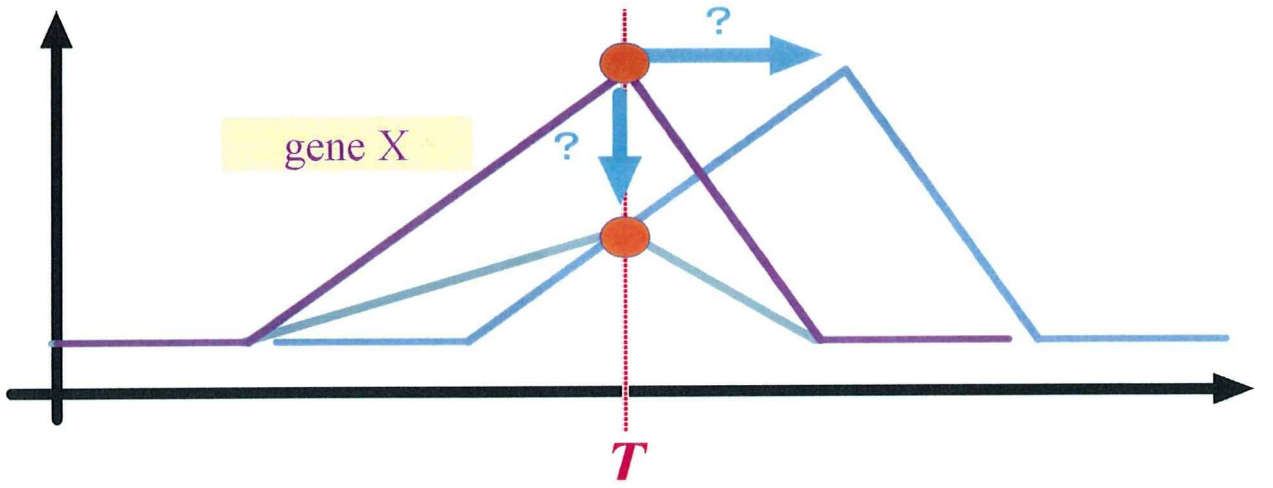
Background3

Problem of doing only by one point in time when paying attention to target genes

Only in the observation of the gene X-expression in the point of time *T*, it is uncertain whether it is a shift of the peak, or the phase, or a compound of both.



61

Construction of database of gene-expression in whole embryo of a wild type from 6.25 to 9.75 dpc

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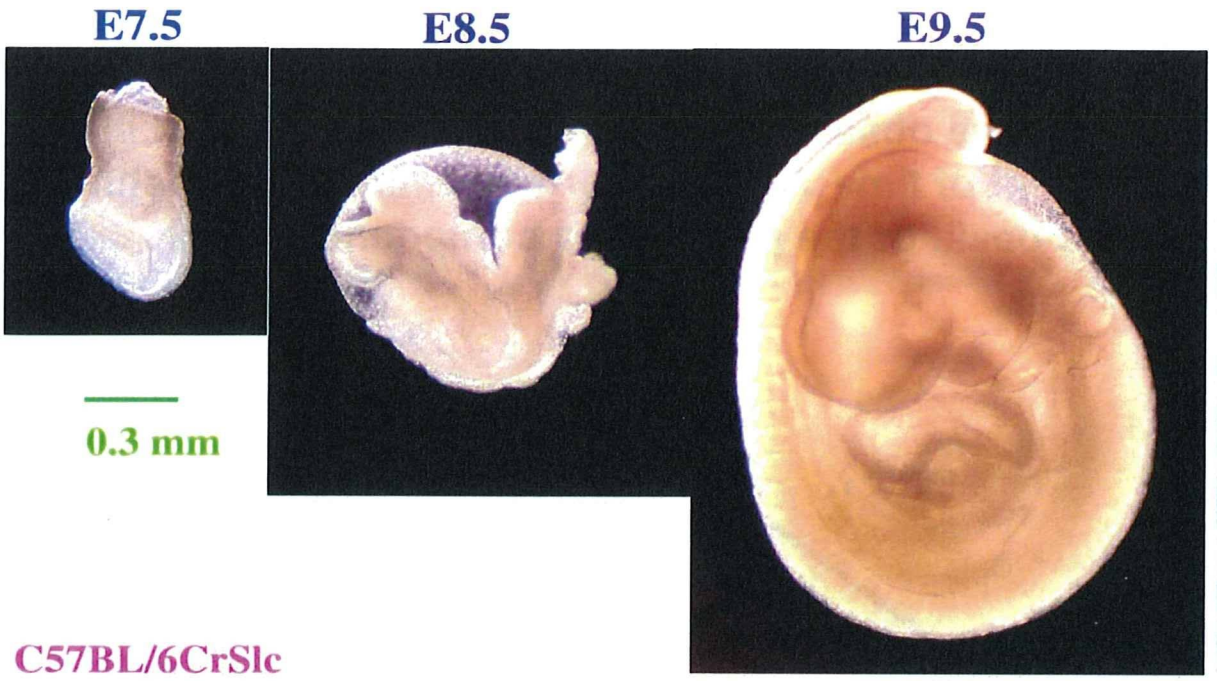
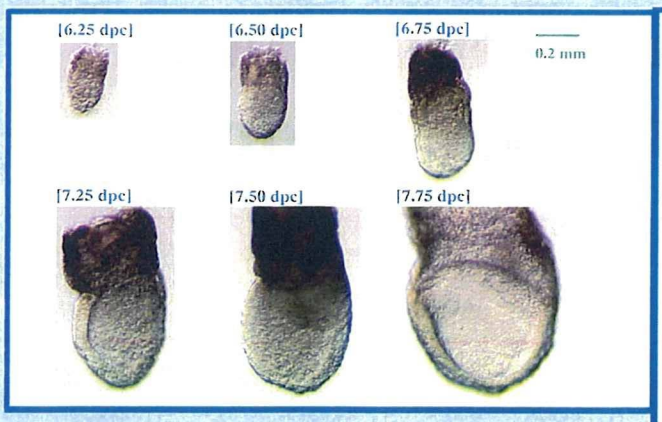
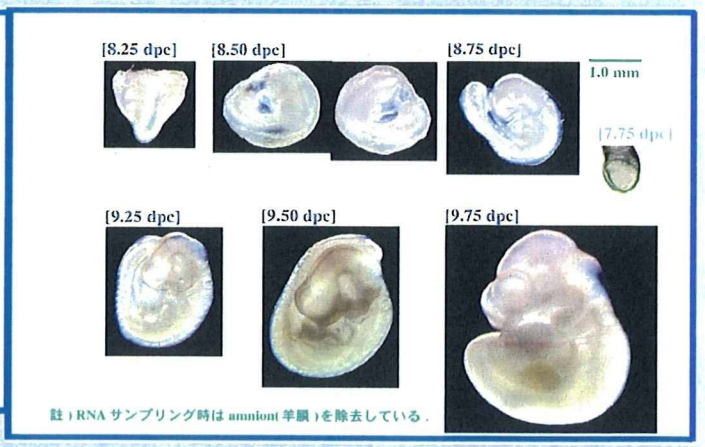


63

12 time point (6.25-9.75 dpc)





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Fetus Percellome Toxicogenomics case study on a model teratogen, cyclopamine

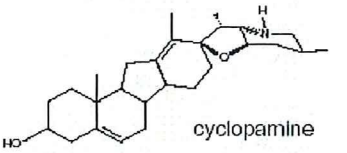
65

Feature of Cyclopamine

-Model teratogen with clear target molecule-

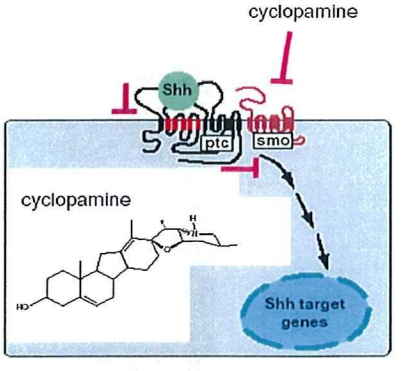
☆ Cyclopamine is a steroidal alkaloid which has been extracted from corn lily (*Veratrum californiucum*)

Inhibits HedgeHog (HH) signaling pathway.
Strict control is critical for correct cell differentiation during embryo genesis. In adults, HH signaling plays role in regulation of stem-cell maintenance and proliferation.



History

- The malformations including cyclopic arose when ewes fed on the plant *Veratorum californicum* (Binns et al., 1963).
- Cyclopamine induced craniofacial anomalies when orally admnistrated to pregnant ewes (Keeler & Binns, 1968).
- Cattle, goats, rabbits and hamsters were susceptible to terato-induction by cyclopamine, however, rats and mice relatively show resistent to the teratogen (Keeler, 1975)
- Cyclopamine inhibits the hedgehog signaling pathway (Hh) by influencing the balance between the active and inactive forms of the Smoothened protein (Smo). (Cooper et al., 1998; Incardona et al., 1998).
- The KO mice of Smo showed cyclopic (Zhangら, 2001).



☆There is no report proven directly that the target molecule of cyclopamine to be embryo's Smo, when the pregnant animal is administrated orally.

66

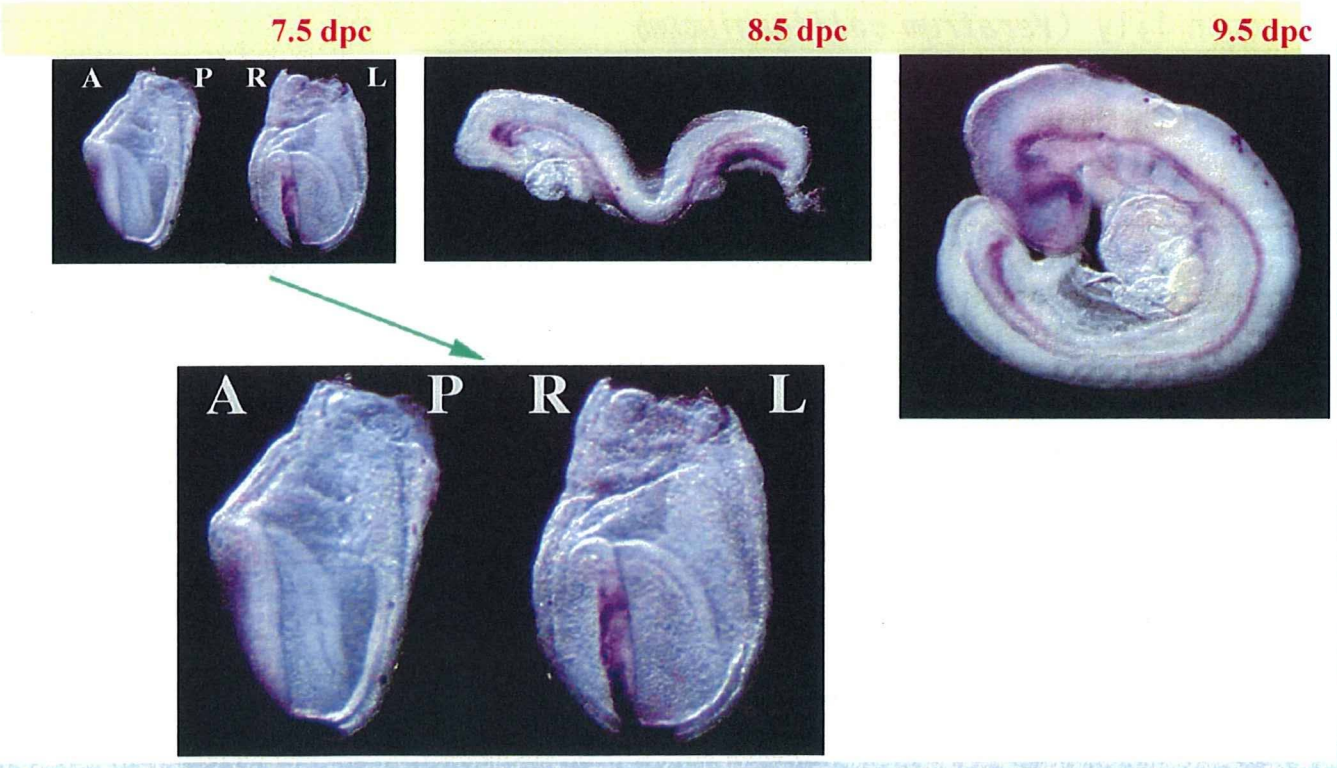
Question

- Is a molecular target of cyclopamine really Smo of the embryo?
- Do other target molecules exist ?

→ Comprehensive gene-expression analysis

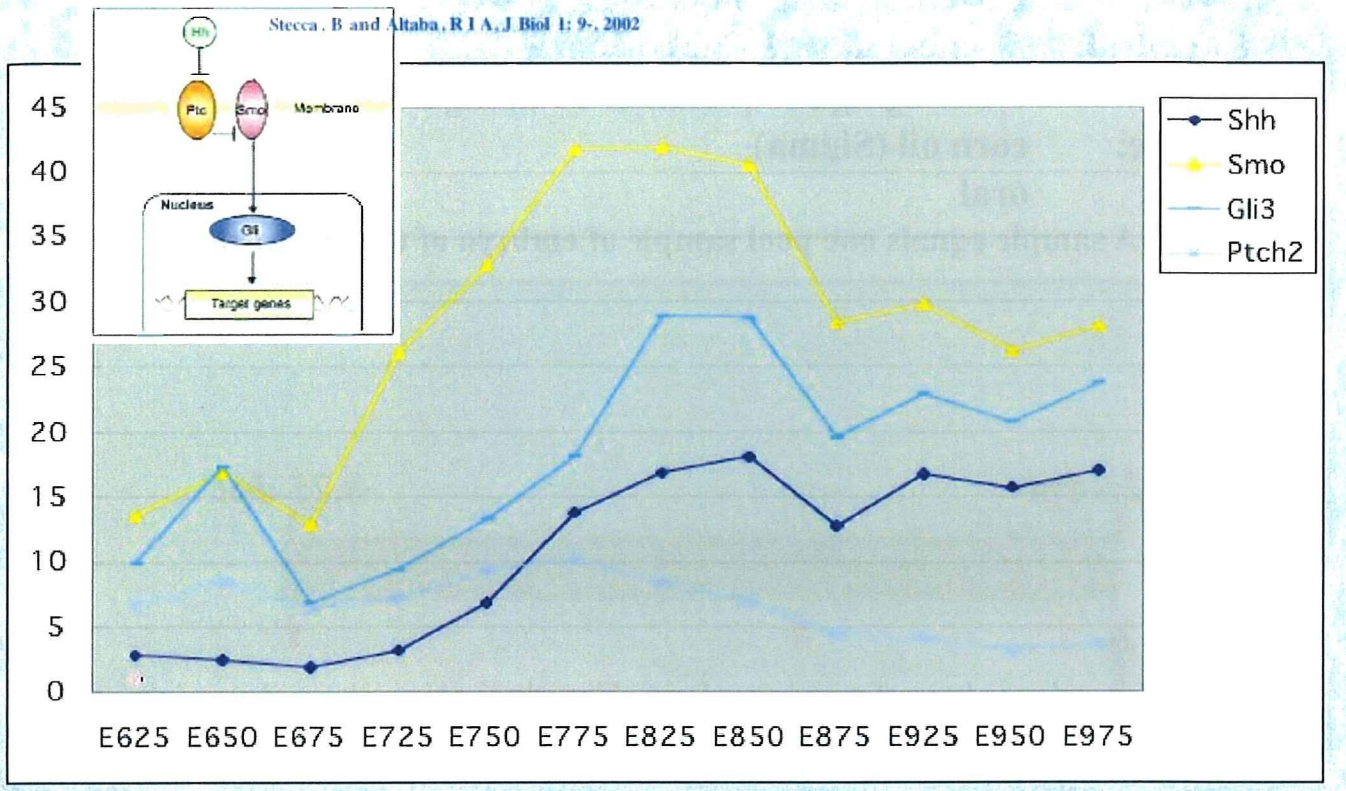
67

The *Shh* expression in mouse embryo from 7.5 to 9.5 dpc



68

The gene-expression profiles which relate to the Shh signaling during 6.25-9.75 dpc in our database using the whole embryo



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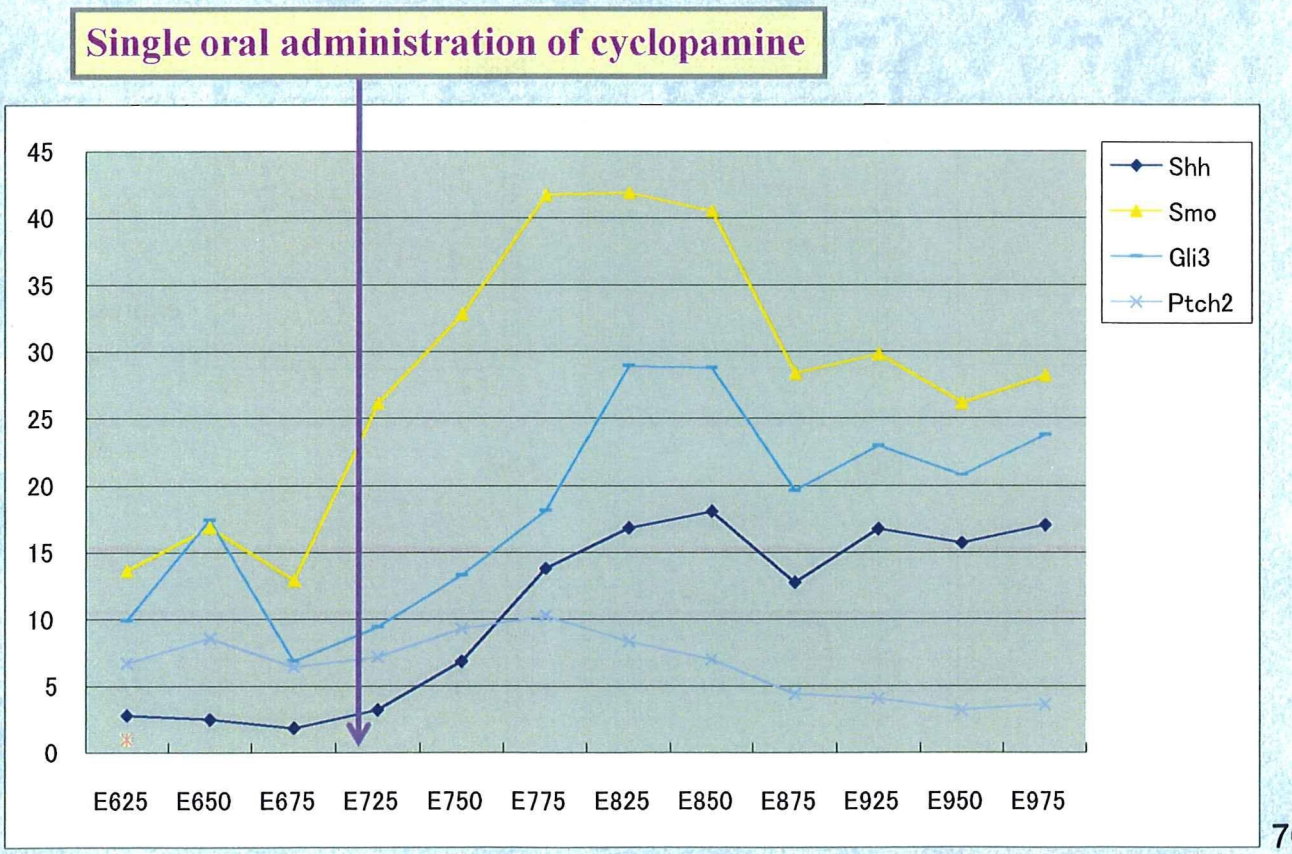
The timing of the cyclopamine administration



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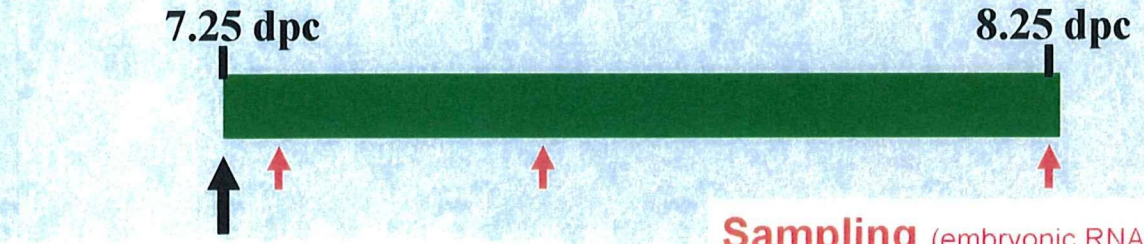
Experiment for the gene-expression analysis

- **Strain:** C57BL/6CrSlc (Japan SLC, Inc.)
- **Chemical:** Cyclopamine (LC laboratories)
- **Dose:** 0, 30 mg/kgBW [volume: 10 ml/kgBW]
- **Vehicle:** corn oil (Sigma)
- **Route:** oral

 # One RNA sample equals one pool sample of embryo of the same litter.



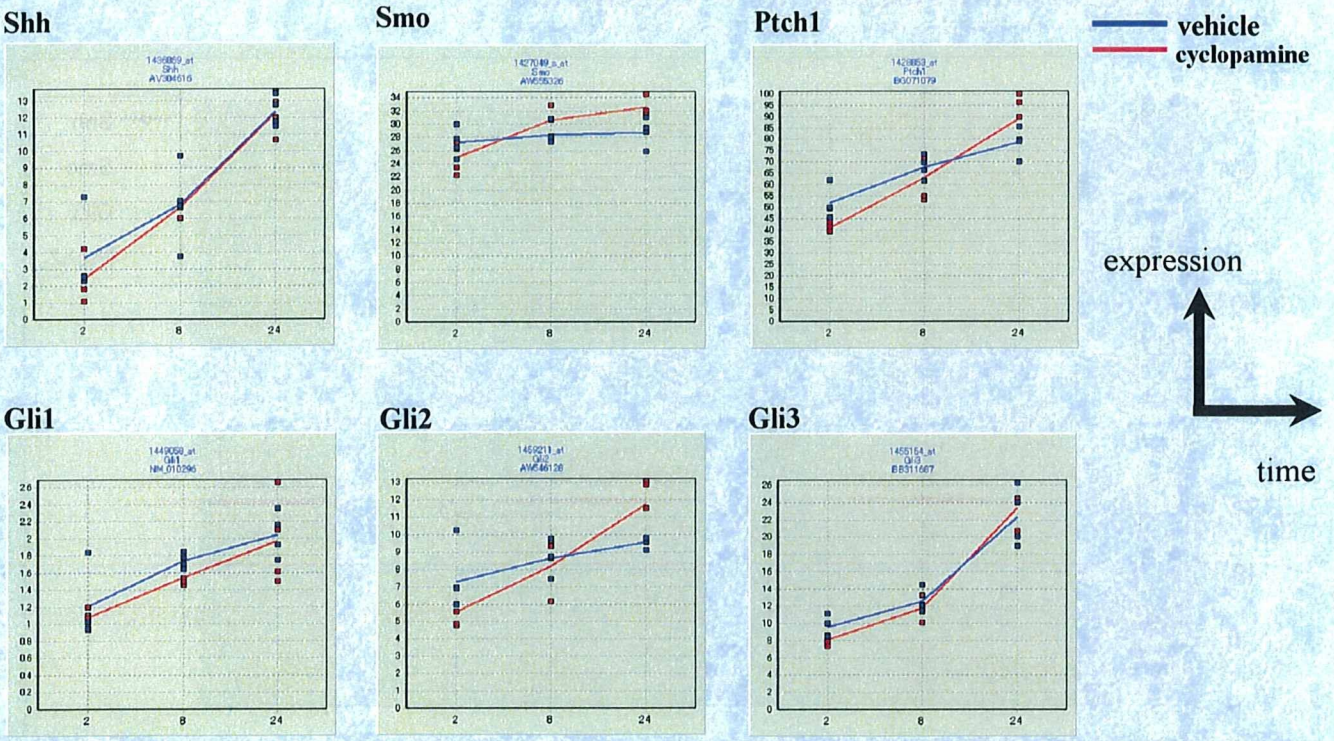
Single oral administration

Sampling (embryonic RNA)

at 2, 8, 24 hours after administration

71

Cyclopamine (30 mg/kg) does not remarkably change the expressions of the genes related to the Shh signaling in the embryo



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Condition setting for analysis

When comparing that in the control group and the treated group,

1) Either copy number of gene expression is larger than that of two.
2) P value of t-test is less than 0.05.
3) The ratio of the mean value of both is larger than that of 1.6.

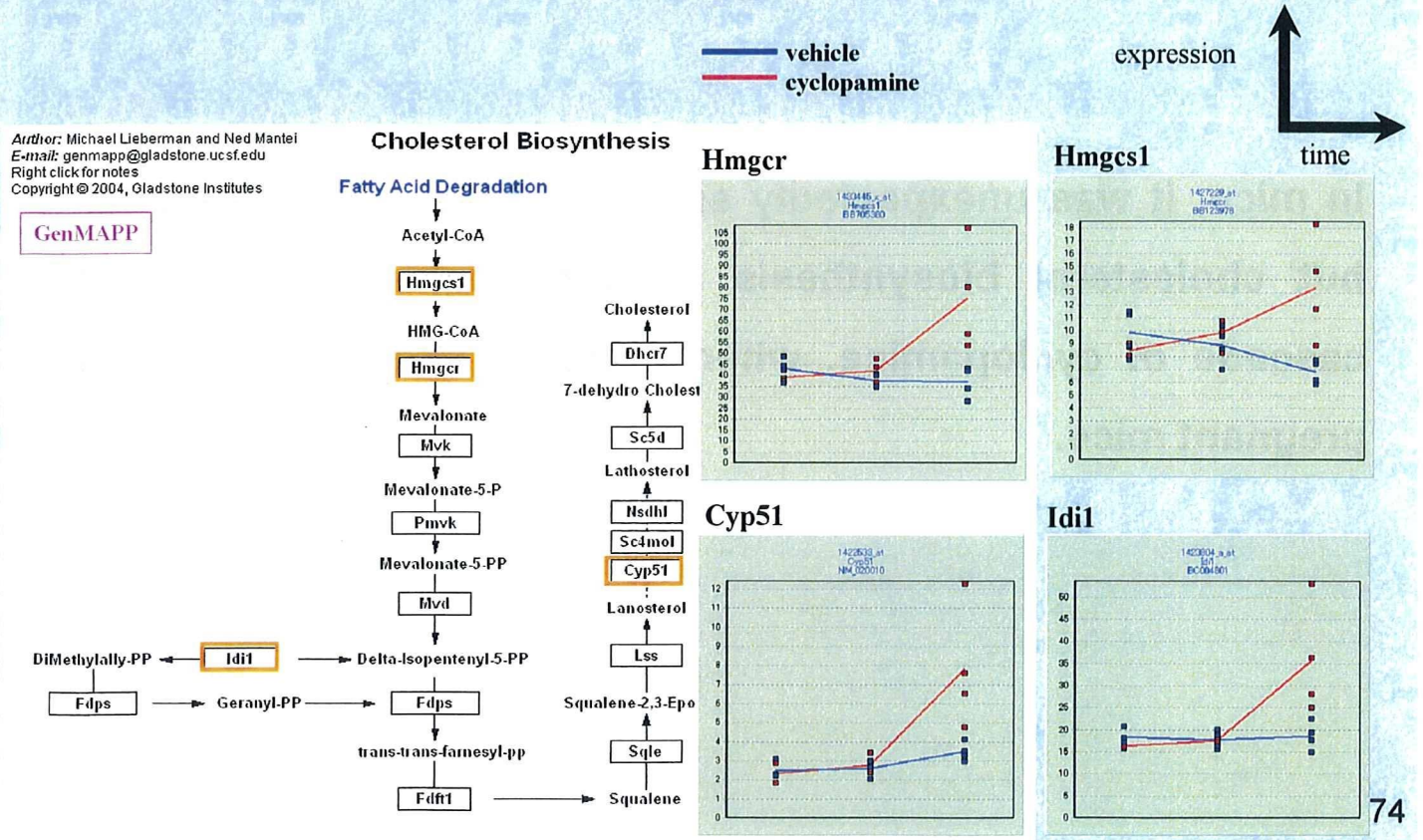
↓

The number of probe set after selected:

- **two hours after administration : 17 P.S.**
- **eight hours after administration : 23 P.S.**
- **twenty four hours after administration : 213 P.S.**

73

Cyclopamine (30 mg/kg) changes the expressions of the genes related to the cholesterol biosynthesis in the embryo



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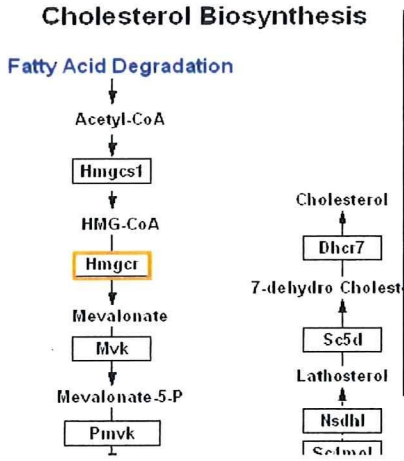
HMG-CoA reductase [Hmgcr] inhibitor "atorvastatin" induces litter resorption (plus maternal death) (300 mg/kgBW)

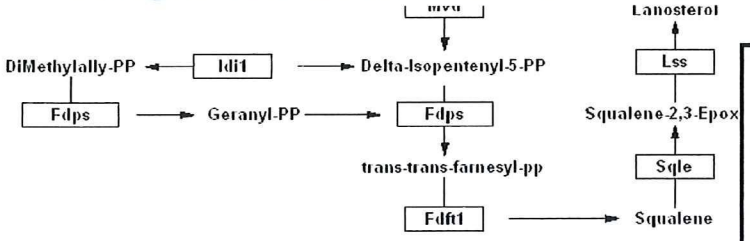
Author: Michael Lieberman and Ned Mantei
E-mail: genmapp@gladstone.ucsf.edu
Right click for notes



- Dostal LA et al., Developmental toxicity of the HMG-CoA reductase inhibitor, atorvastatin, in rats and rabbits. *Teratology* 50: 387-394, 1994.

- Henck JW et al., Pre- and postnatal toxicity of the HMG-CoA reductase inhibitor atorvastatin in rats. *Toxicol Sci* 41: 88-99, 1998.

Hmgcr-KO mice shows embryonic lethality (8.5 dpc)



- Ohashi K et al., Early embryonic lethality caused by targeted disruption of the 3-hydroxy-3-methylglutaryl-CoA reductase gene. *J Biol Chem* 278: 42936-42941, 20030

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Thus,

In mice, it was unexpectedly suggested that not Shh signal, but cholesterol biosynthesis system might be the target cascade of cyclopamine, when administrating orally to the pregnant mice.

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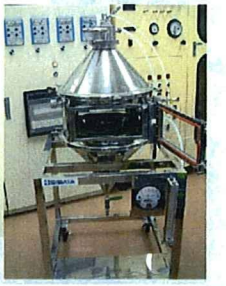
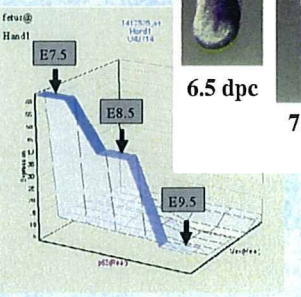
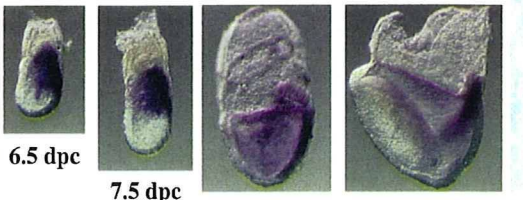
Percellome Project (mouse)

@Div. Cellular and Molecular Toxicology/ BSRC/ NIHS

- TTG1: 2003~ (Adult mouse)
  - –single exposure, oral. liver 100 chem
  - –gene knockout mouse
- TTG2: 2006~ (Adult mouse)
  - –repeated exposure, oral
  - –multi-organ
  - –high-through put *in situ* hybridization
- **TTG3: 2009~** (Adult mouse)
  - –Informatics
- ITG: 2004~ (Adult mouse)
  - –inhalation (Lung & Liver), low dose level
  - –2hr exposure, 6hr, 22hr,
- FTG: 2003~ (Fetus)
  - –Developmental TG
  - –Various developmental stages
  - –Gene knockout mouse embryo
  - –[Phylogenic development (PDTG)]
- Food TG:
- Combine effect TG:
- Others







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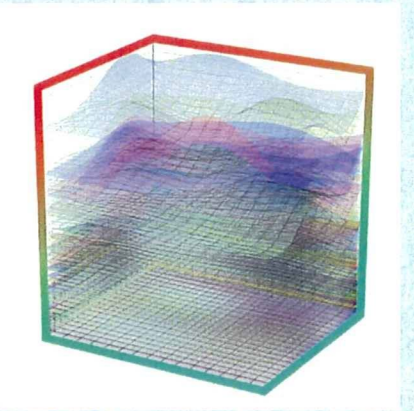
Development of Percellome (2001~)

Jun Kanno, MD, PhD
Katsuhide Igarashi, PhD
Ken-ichi Aisaki, MD, PhD
Atsushi Ono, PhD
Tomoko Ando, Ms
Noriko Moriyama, Ms
Yuko Kondo, Ms
Yuko Nakamura, Ms
Maki Abe, Ms



Percellome (Millefeuille) (2003~)

Ken-ichi Aisaki, MD, PhD
Katsuhide Igarashi, PhD
Noriyuki Nakatsu, PhD
Yukio Kodama, DVM
Tomoko Ando, Ms
Noriko Moriyama, Ms
Yuko Kondo, Ms
Yuko Nakamura, Ms
Maki Abe, Ms
Kenta Yoshiki, Mr
Nae Matsuda, DVM
Chiyuri Aoyagi, Ms
Kouichi Morita, Mr
Ayako Imai, Ms
Shinobu Watanabe, Ms
Yukio Ogawa, DVM (Inhalation)
Satoshi Kitajima DVM, PhD (Fetus)
Kentaro Tanemura DVM, PhD

NIHS TGP (with 17 Pharm) startup group (~summer 2002)

Akihiko Hirose	Risk Assess/ BSRC/ NIHS
Takayoshi Suzuki	Mutagen/ BSRC/ NIHS
Makoto Shibutani	Path/ BSRC/ NIHS
Katsuhide Igarashi	Tox/BSRC/NIHS
Atsushi Ono	Tox/BSRC/NIHS
Ken-ichi Aisaki	Tox/BSRC/NIHS
Jun Kanno	Tox/BSRC/NIHS

Millefeuille Softwares

Ken-ichi Aisaki, MD, PhD

IT collaboration

NTT COMWARE
with Teradata, NCR

Grants

Ministry of Health, Labor, and Welfare (MHLW) Grant-in-Aid H21-kagaku-ippan-001, H18-kagaku-ippan-001, H17-kagaku-003, H15-kagaku-002, H14-Toxico-001, H13-seikatsu-012, & MOE

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Bioinformatics Tools

- **MicroArray Quality Control (MAQC)**
- MAQC Guidance Document and SOPs
- MAQC Meetings
- MAQC Participating Organizations
- MAQC Presentations
- MAQC Publications
- MAQC Teleconferences
- MAQC Timeline
- MAQC Working Groups

Resources for You

- About the National Center for Toxicological Research
- Executive Summary of MAQC-I (PDF - 43KB)
- Summary - MAQC Data Sets (PDF - 109KB)
- Toxicoinformatics

MicroArray Quality Control (MAQC)

MAQC Timeline: Get e-mail updates

Nature Biotechnology
September 2006 issue featuring MAQC-I

MAQC-I
MAQC-II
MAQC-III (also known as SEQC)

Microarrays and next-generation sequencing represent core technologies in pharmacogenomics and toxicogenomics; however, before these technologies can successfully and reliably be used in clinical practice and regulatory decision-making, standards and quality measures need to be developed. The MAQC project is helping improve the microarray and next-generation sequencing technologies and foster their proper applications in discovery, development and review of FDA regulated products. Everyone is invited to participate in the MAQC project.

MAQC-I

The first phase of the MAQC project (MAQC-I) aims to:

- provide quality control (QC) tools to the microarray community to avoid procedural failures
- develop guidelines for microarray data analysis by providing the public with large reference datasets along with readily accessible reference RNA samples
- establish QC metrics and thresholds for objectively assessing the performance achievable by various microarray platforms
- evaluate the advantages and disadvantages of various data analysis methods

MAQC-I involves six FDA Centers, major providers of microarray platforms and RNA samples, EPA, NIST, academic laboratories, and other stakeholders. Two human reference RNA samples have been selected, and differential gene expression levels between the two samples have been calibrated with microarrays and other technologies (e.g., QRT-PCR). The resulting microarray datasets have been used for assessing the precision and cross-platform/laboratory comparability of microarrays, and the QRT-PCR datasets enabled evaluation of the nature and magnitude of any systematic biases that may exist between microarrays and QRT-PCR. The availability of the well-characterized RNA

Spotlight

- Nature Biotechnology - MicroArray Quality Control (MAQC) project

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samples combined with the resulting microarray and QRT-PCR datasets, which have been made readily accessible to the scientific community, allow individual laboratories to more easily identify and correct procedural failures.

Results from the MAQC-I were published in six research papers in September 2006 in *Nature Biotechnology*

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MAQC-II

The second phase of the MAQC project (MAQC-II) aims to:

- assess the capabilities and limitations of various data analysis methods in developing and validating microarray-based predictive models
- reach consensus on the "best practices" for development and validation of predictive models based on microarray gene expression and genotyping data for personalized medicine

Thirty-six teams developed classifiers for 13 endpoints—some easy, some difficult to predict, from six relatively large training data sets. These analyses collectively produced >18,000 models that were challenged by independent and blinded validation sets generated for MAQC-II. The cross-validated performance estimates for models developed under good practices are predictive of the blinded validation performance. The achievable prediction performance is largely determined by the intrinsic predictability of the endpoint, and simple data analysis methods often perform as well as more complicated approaches. Multiple models of comparable performance can be developed for a given endpoint and the stability of gene lists correlates with endpoint predictability. Importantly, similar conclusions were reached when >12,000 new models were generated by swapping the original training and validation sets.

Over 10 manuscripts from the MAQC-II have been submitted to Nature Biotechnology for peer review.

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MAQC-III (also known as SEQC)

The third phase of the MAQC project (MAQC-III), also called Sequencing Quality Control (SEQC), aims at assessing the technical performance of next-generation sequencing platforms by generating benchmark datasets with reference samples and evaluating advantages and limitations of various bioinformatics strategies in RNA and DNA analyses.

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RNA Samples

The availability of the calibrated RNA samples combined with the resulting microarray and QRT-PCR datasets, which will be made readily accessible to the microarray community, will allow individual laboratories to more easily identify and correct procedural failures.

Contact Information

Please address any other questions and suggestions to Dr. Leming Shi at 870-543-7387 or Leming.Shi@fda.hhs.gov.

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MAQC Timeline

Get e-mail updates when this information changes.

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- **February 11, 2005:** Phase I of the MAQC project (MAQC-I) on microarray technical performance launched
- **June 5, 2006:** MAQC-I manuscripts submitted
- **September 8, 2006:** MAQC-I results published in September 2006 issue of *Nature Biotechnology*
- **September 8, 2006:** MAQC-I datasets made publicly available
- **September 21, 2006:** MAQC-II on predictive models (signatures) launched
- **August 28, 2007:** "Pharmacogenomic Data Submissions — Companion Guidance" released
- **December 16-17, 2008:** MAQC-III (or SEQC) on next-generation sequencing launched
- **March 2009:** MAQC-II manuscripts submitted
- **September 2009:** MAQC-II results published
- **December 2009:** MAQC-III manuscripts submitted
- **May 2010:** MAQC-III results planned for publication

Contact Information

Please address questions and suggestions about the MicroArray Quality Control project to the MAQC Coordinator, Dr. Leming Shi, National Center for Toxicological Research, at 870-543-7387 or Leming.Shi@fda.hhs.gov.

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