

## A major prerequisite is that

the Toxicology must be prepared for any unexpected or unpredictable responses.

Thus, the approach must be comprehensive.

5

We adopted a whole-genome cDNA microarray system for a **comprehensive** monitoring of the transcriptome,

and launched the **Percellome Toxicogenomics Project** to develop the **comprehensive** gene regulatory network database and its informatics for the mechanism-based predictive toxicology.

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# Percellome Method

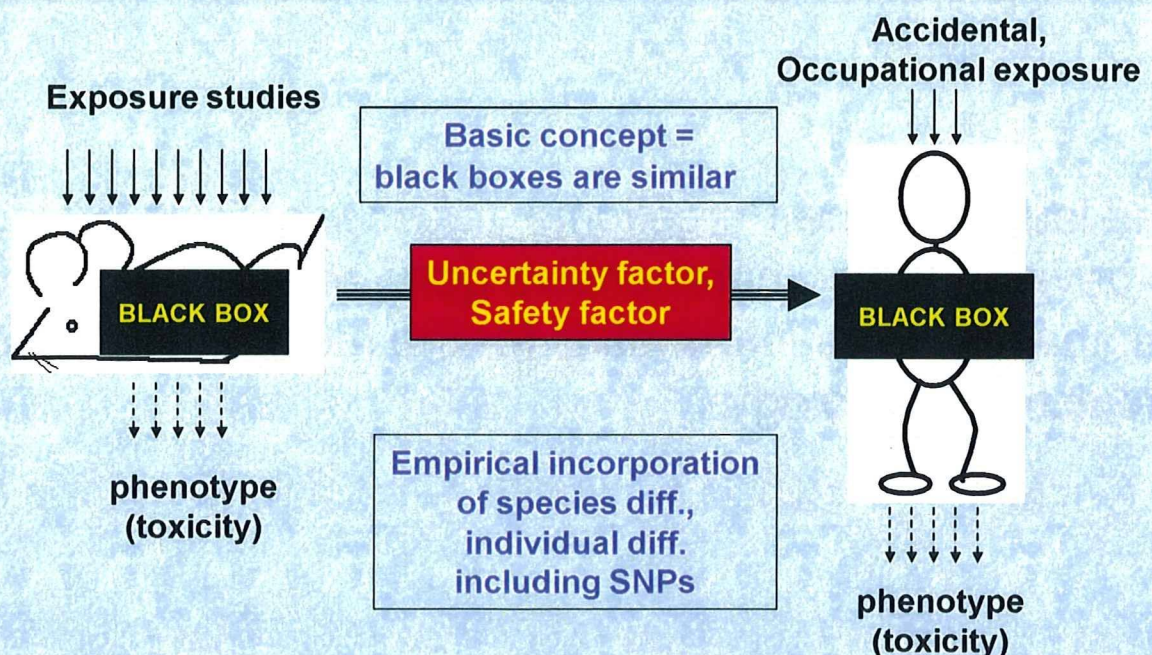
Obtain mRNA data in  
“copy numbers of mRNA per cell”  
from microarrays and Q-RT-PCR



**easy and direct data comparison  
among studies and even different organs**

7

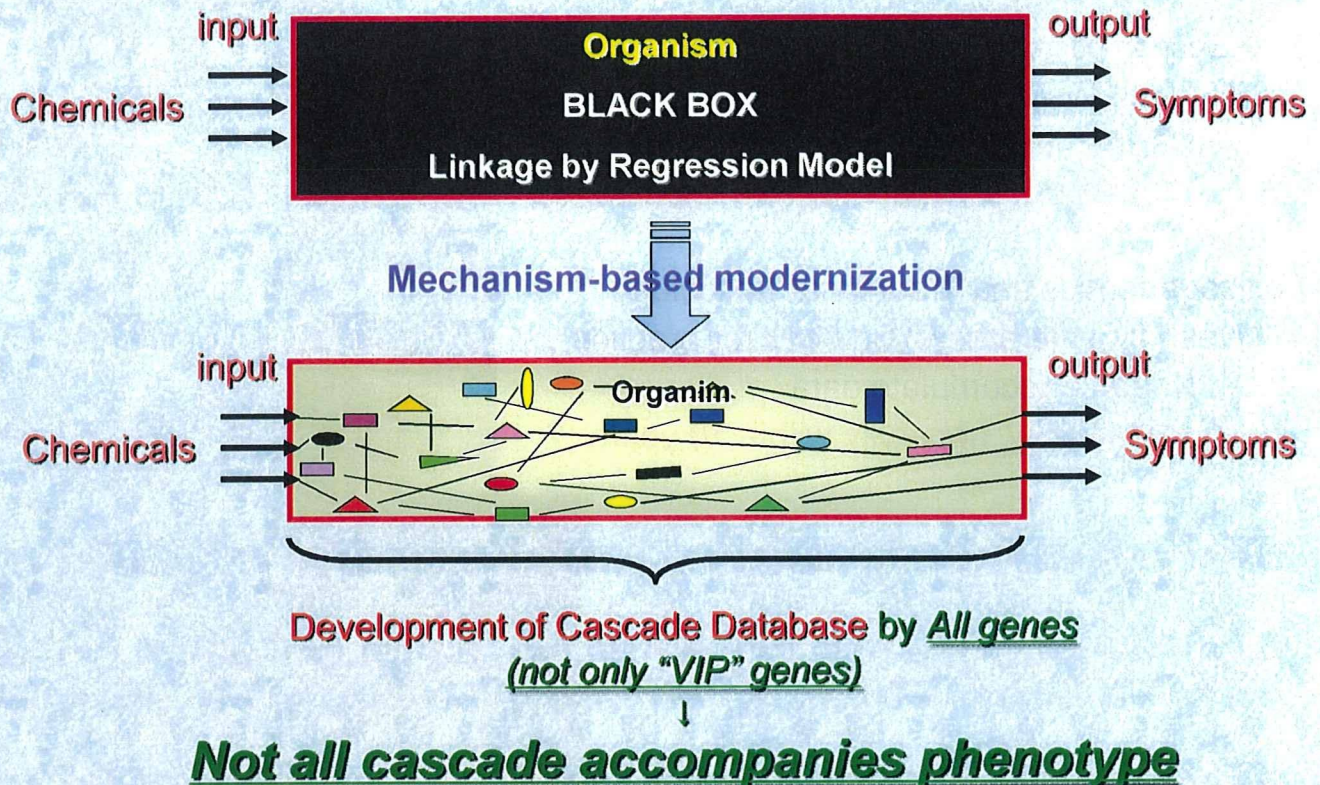
## Current status



8



# Why Percellome, why Phenotype-Independent?



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## Percellome Projects

### Aim:

To Develop **Gene Cascade Database** by Phenotype-Independent Approach for Predictive Toxicology

### Ultimate Goal:

To Develop "virtual mouse, virtual human *in silico*"

### Tentative Goal:

High-Resolution, Mechanism-based Toxicology to reinforce Traditional Toxicology

analogy: Electron Microscopy and Light Microscopy

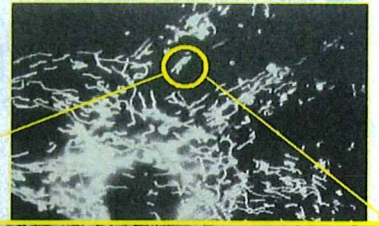
The basic researches for the establishment of our toxicogenomics are essential in a way similar to making new textbooks or atlases of electron microscopy for its practical applications.

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Electron Microscopy and Light Microscopy  
Needed to write a new text book for practice  
Needed to accumulate data  
..... it took 10~20 years to write text books

Toxicogenomics and Traditional Toxicology  
Need to write a new text book for practice  
Need to accumulate data  
..... Underway, hopefully within 10 years !?



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## **Percellome Project**

is developed for

**Phenotype-Independent**

**Toxicogenomics**

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Methodology article

Open Access

"Per cell" normalization method for mRNA measurement by quantitative PCR and microarrays

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Email: Jun Kanno\* - kanno@nihs.go.jp; Ken-ichi Aisaki - aisaki@nihs.go.jp; Katsuhide Igarashi - igarashi@nihs.go.jp; Noriyuki Nakatsu - n-nakatsu@nihs.go.jp; Atsushi Ono - atsushi@nibio.go.jp; Yukio Kodama - kodama@nihs.go.jp; Taku Nagao - nagao@nihs.go.jp

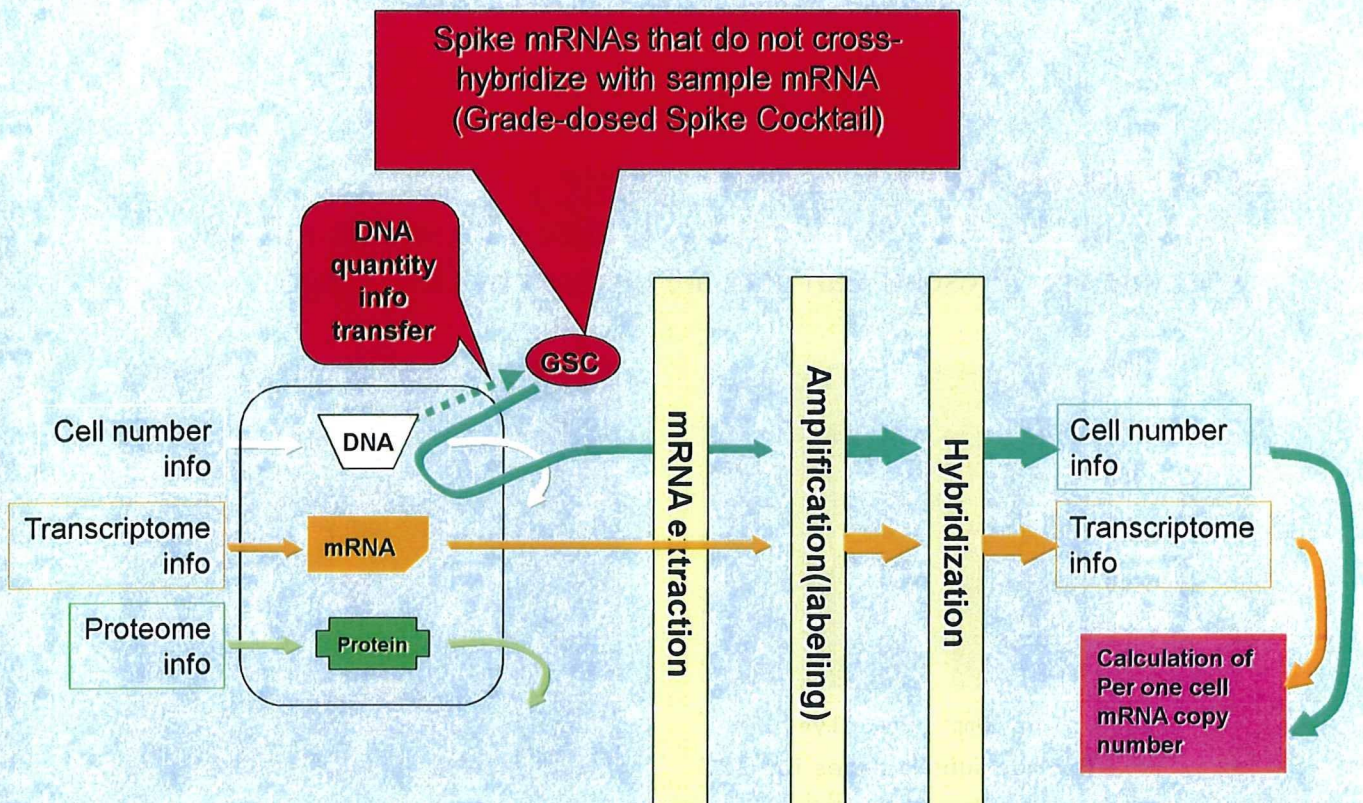
\* Corresponding author †Equal contributors

Open Access

on line journal: BMC Genomics 7(1): 64 , 2006

PMID: 16571132

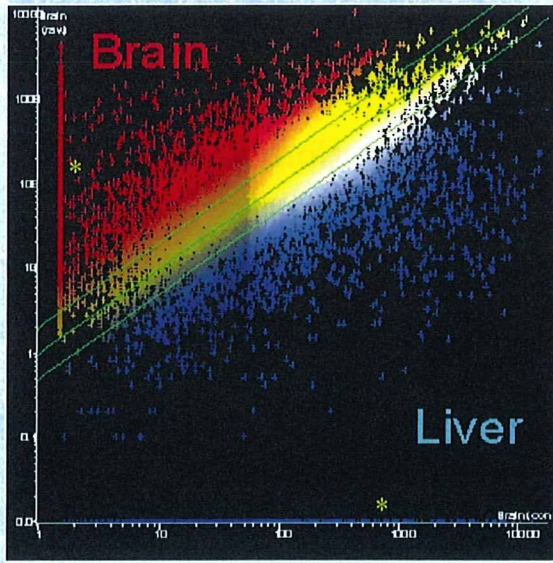
13



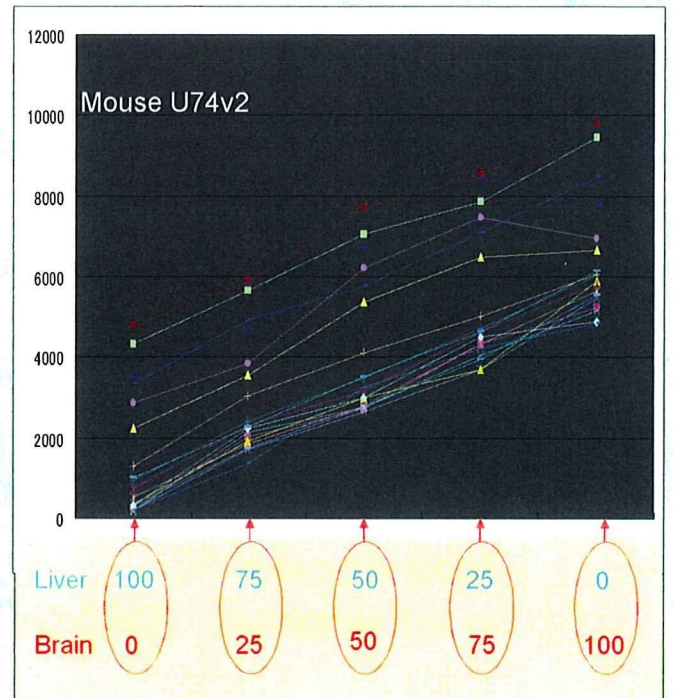
14



## Platform Performance required for Percellome Normalization = Dose-response range and linearity



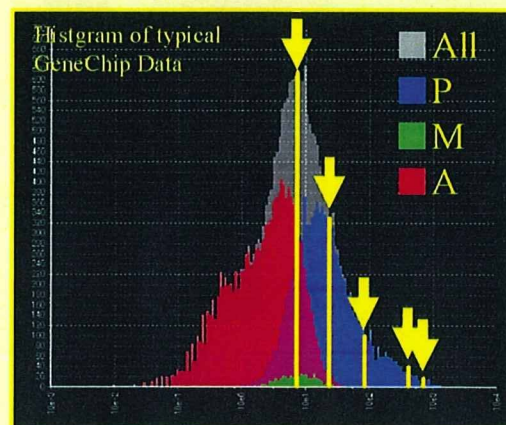
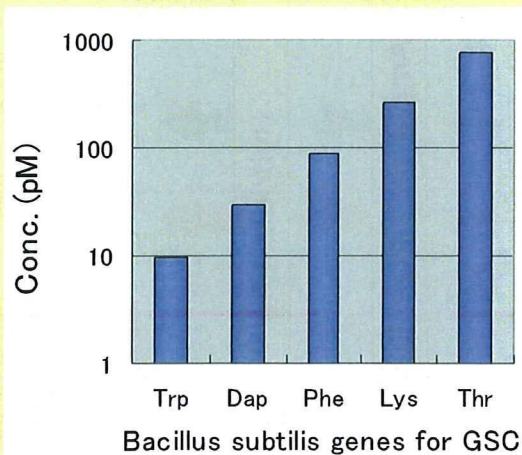
LBM (Liver-Brain-Mix) for verification  
of Dose-response capability of the system



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### \* Grade-dosed Spike Cocktail (GSC)

- Spike mRNAs that do not cross-hybridize with sample mRNA
- Affymetrix “Present” call data are covered by GSC range

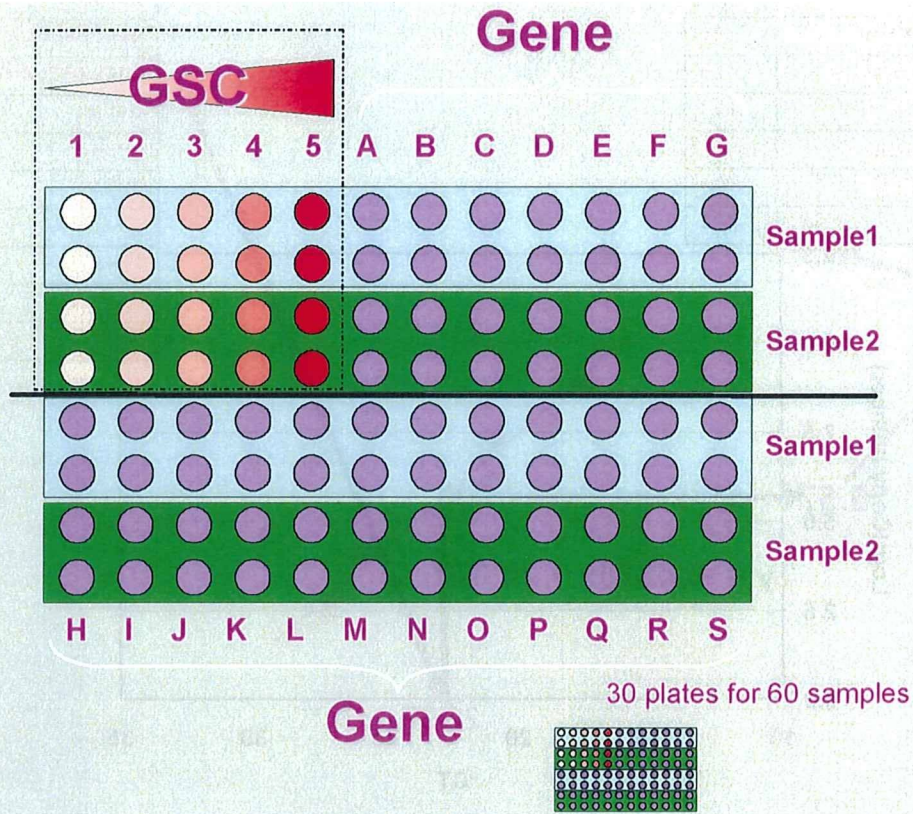


16



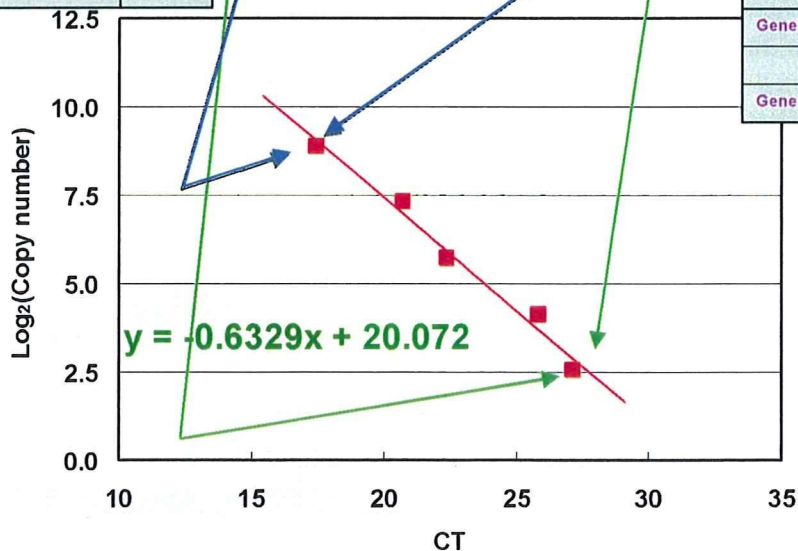
# Percellome Quantitative-PCR

(by ABI PRISM 7900HT / SYBR Green)



## Percellome Q-PCR normalization

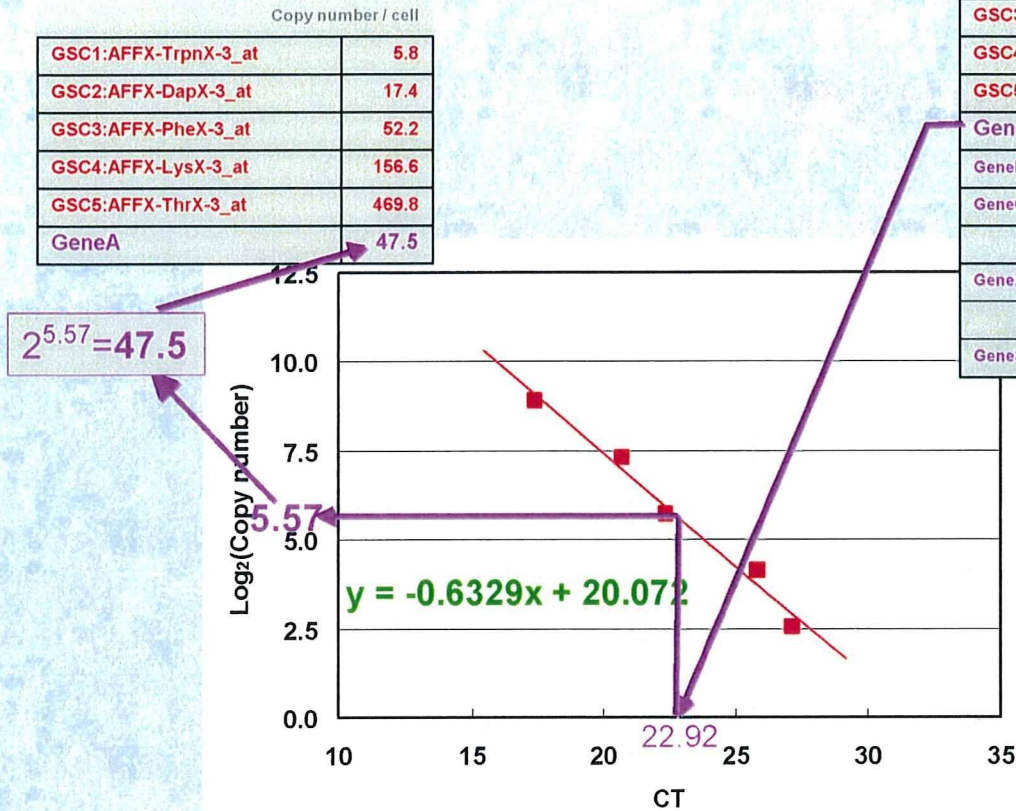
Copy number / cell	
GSC1:AFFX-TrpnX-3_at	5.8
GSC2:AFFX-DapX-3_at	17.4
GSC3:AFFX-PheX-3_at	52.2
GSC4:AFFX-LysX-3_at	156.6
GSC5:AFFX-ThrX-3_at	469.8
GeneA	???



CT	
GSC1:AFFX-TrpnX-3_at	27.13
GSC2:AFFX-DapX-3_at	25.85
GSC3:AFFX-PheX-3_at	22.38
GSC4:AFFX-LysX-3_at	20.72
GSC5:AFFX-ThrX-3_at	17.41
GeneA	22.92
GeneB	22.34
GeneC	24.47
GeneX	28.44
GeneS	22.30



# Percellome Q-PCR normalization



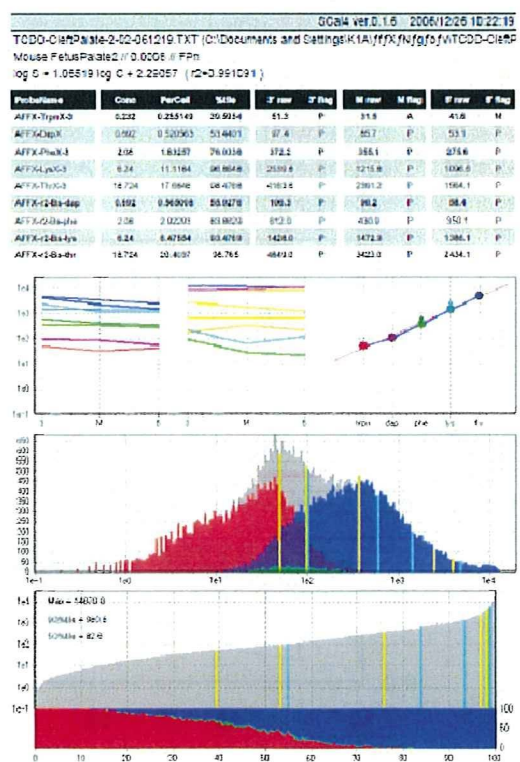
19

## Percellome Normalization, calculation and QC (for microarray)

SCal4

Affymetrix GeneChip  
Mouse 430 v2  
(Rat, Human, Xenopus)

Exon Array  
(human and mouse)  
now under trial



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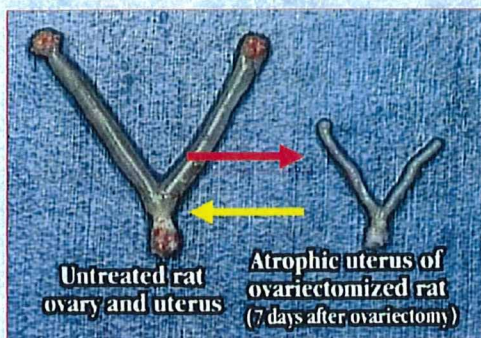


# Some Features of Percellome Method

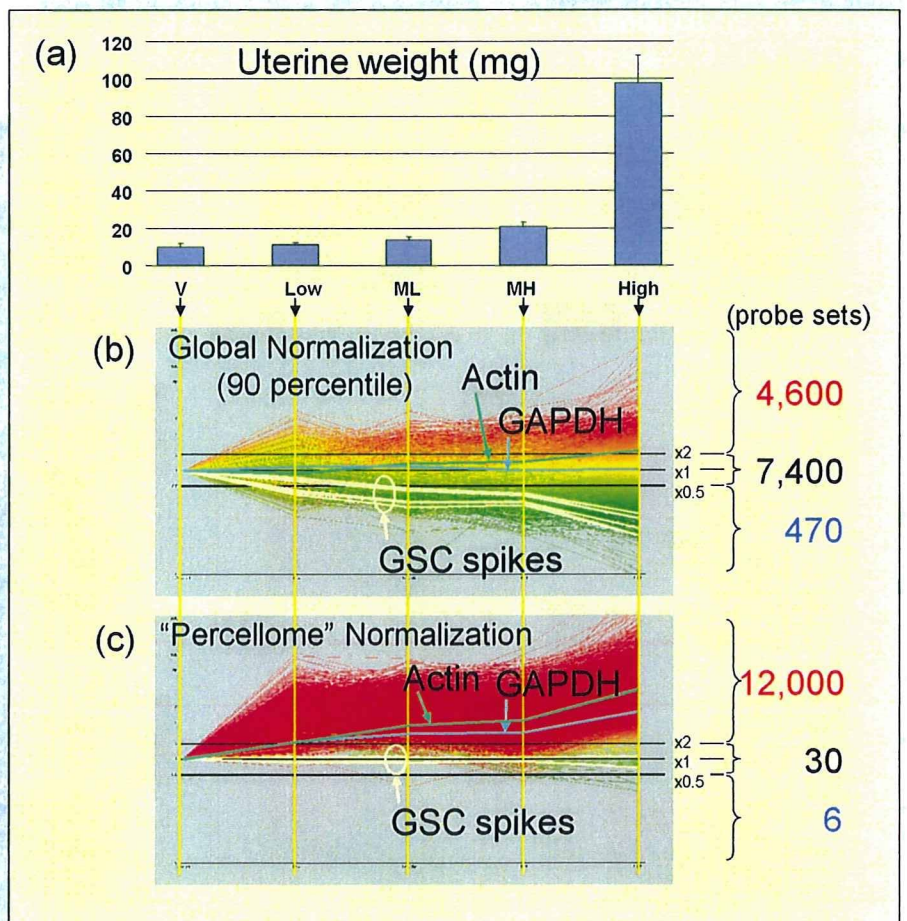
21

## Profile-independent normalization

### Ovariectomy

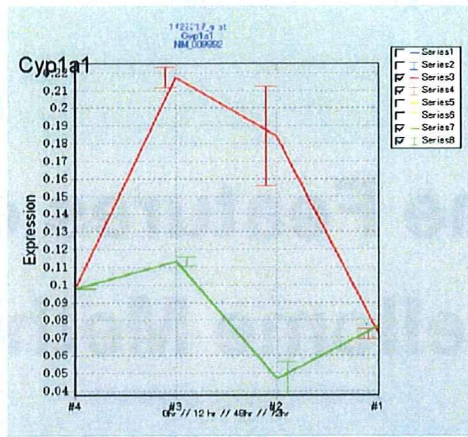


Chemical (estrogenic) effect

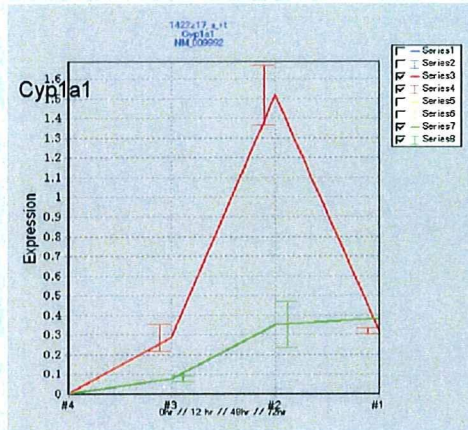




# In vitro Experiment



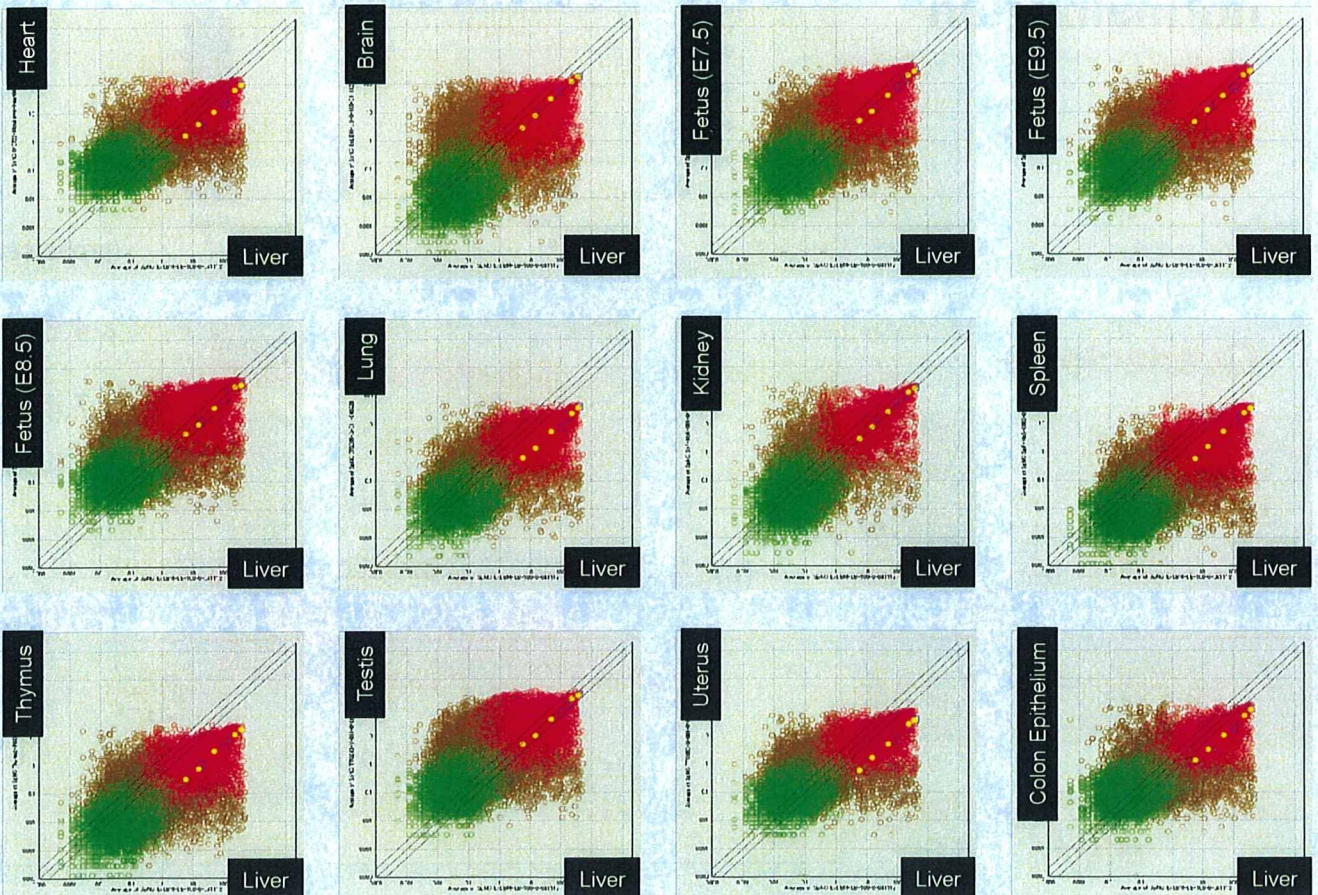
Global Normalization



Percellome

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## Direct Comparison among Organs

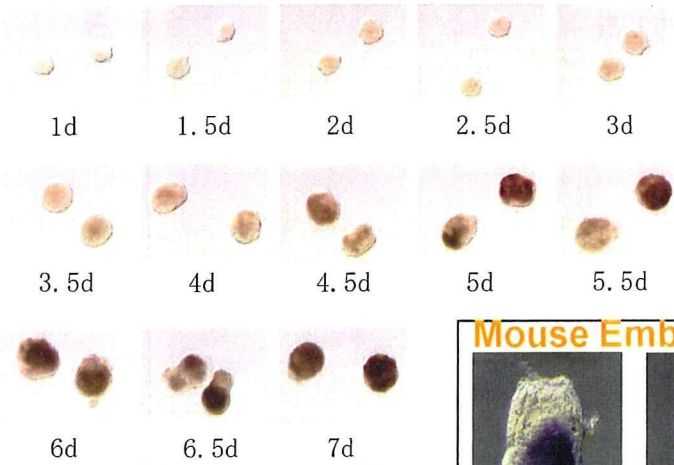


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# Fetus (Developmental) Toxicogenomics -Percellome-

## Embryoid Body

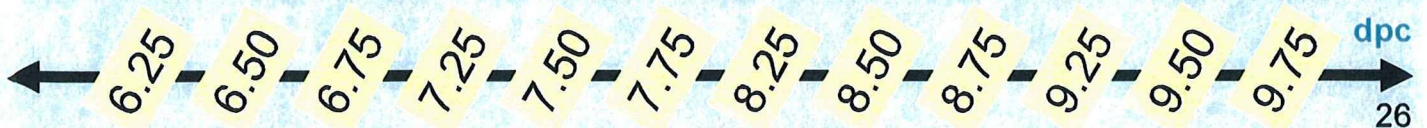
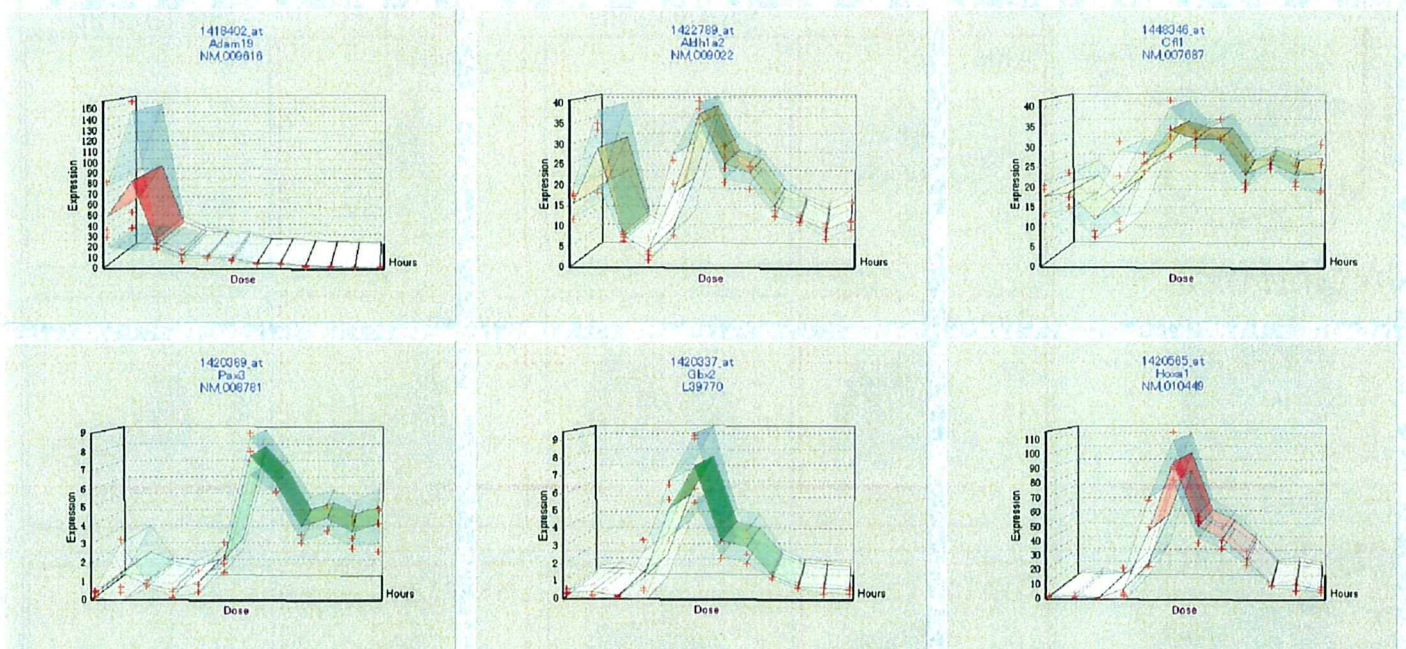


## Mouse Embryo



Dr. Satoshi Kitajima

## FTG Percellome database: whole embryo early development stages (6.25 ~ 9.75 dpc)

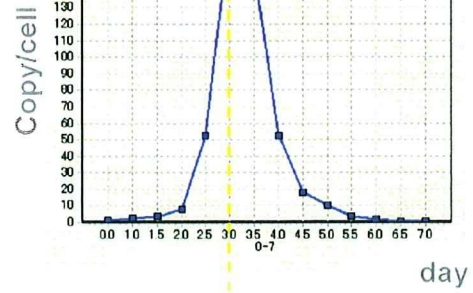
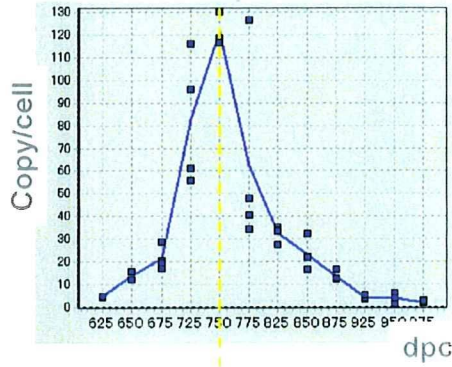




## Whole Embryo

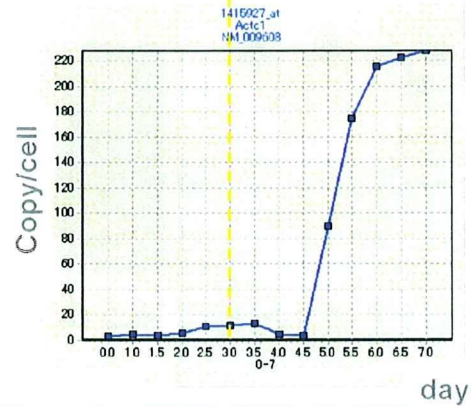
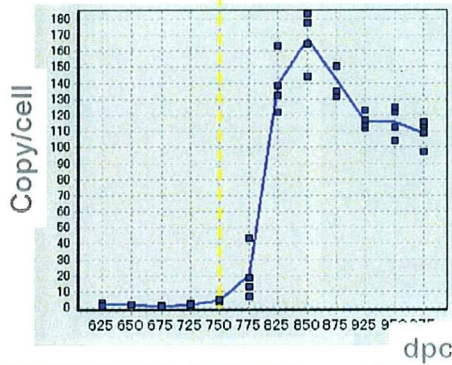
## Embryoid Body

Brachyury



Brachyury

Cardiac actin

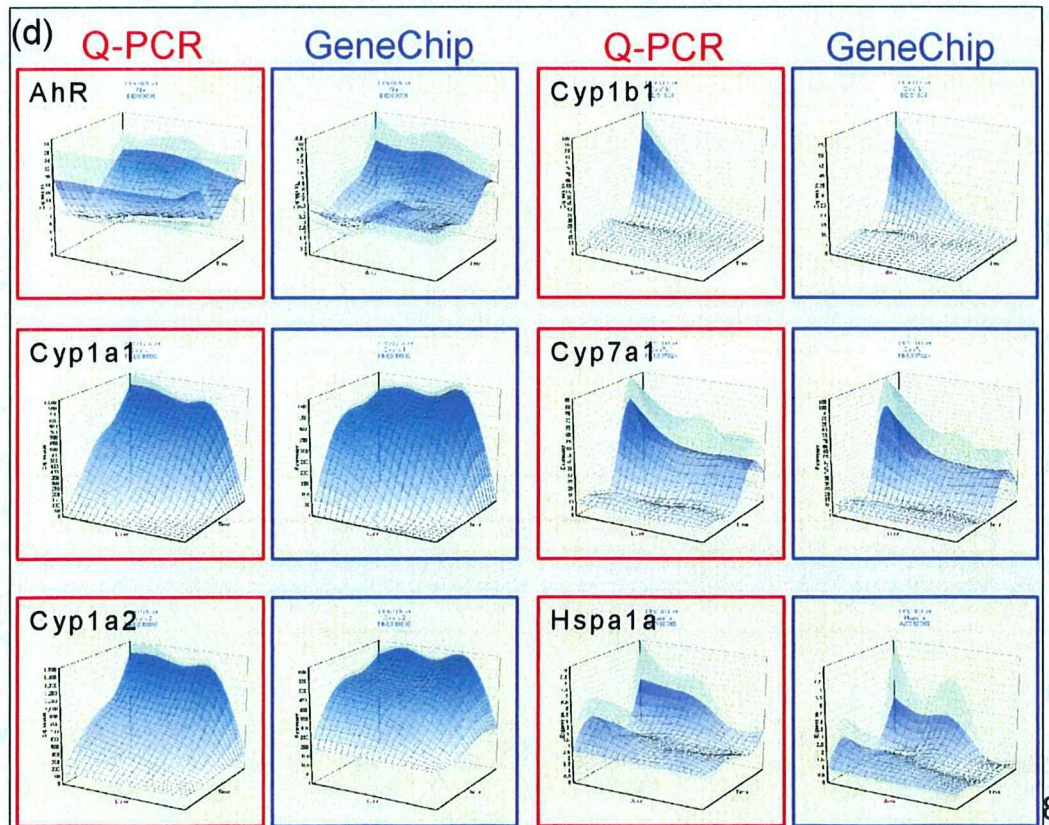
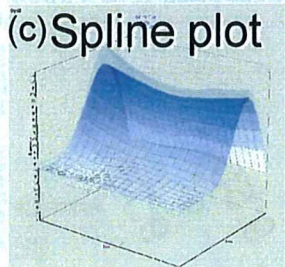
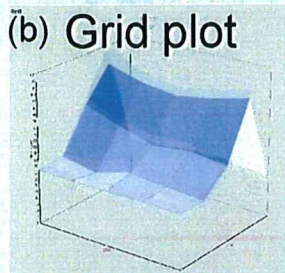
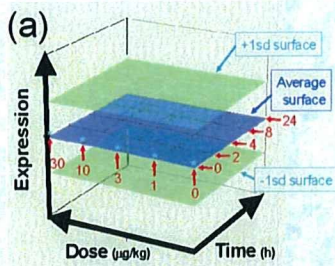


Cardiac actin

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## Percellome Q-PCR versus GeneChip

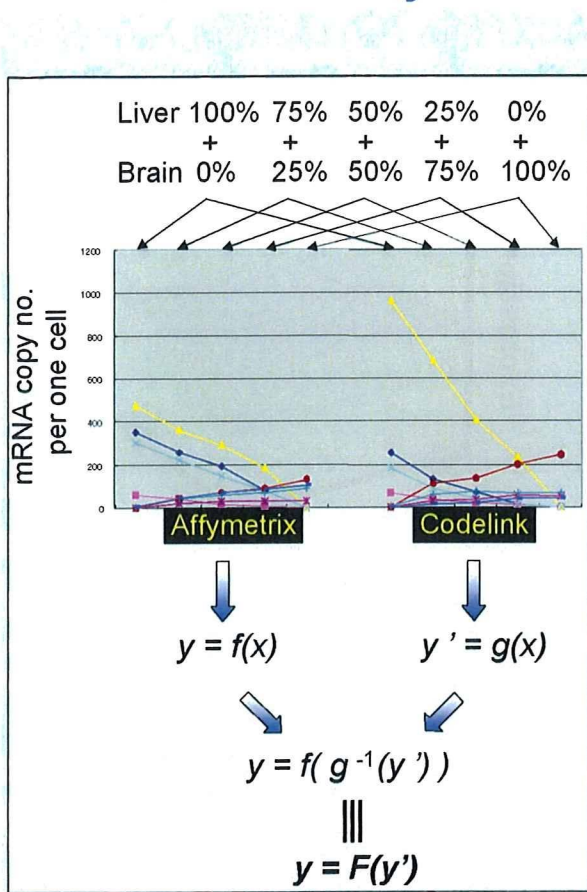
TCDD /// 5x4 n=3, 48 sample /// GSC(5)+19 primer pair set1



8

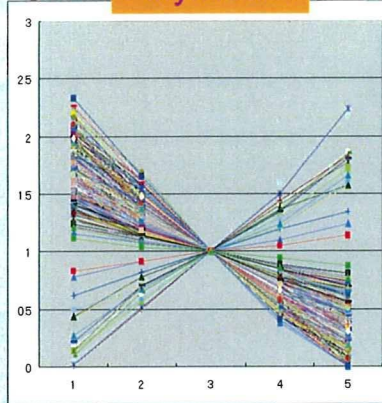


## Data Conversion by Percellome

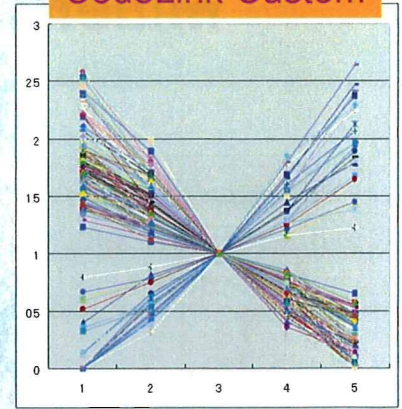


To 250  
 High linearity  
 probesets  
 ( $R^2$  closest to 1)

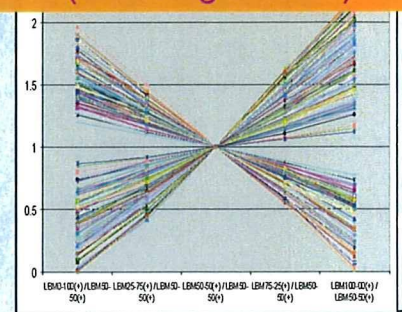
Affymetrix



CodeLink Custom



Agilent Custom  
 (44k single color)



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Up to now,

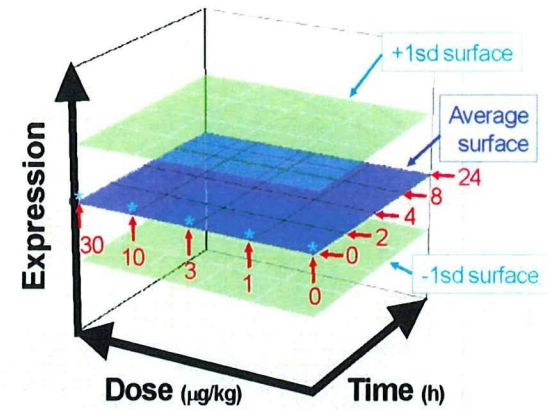
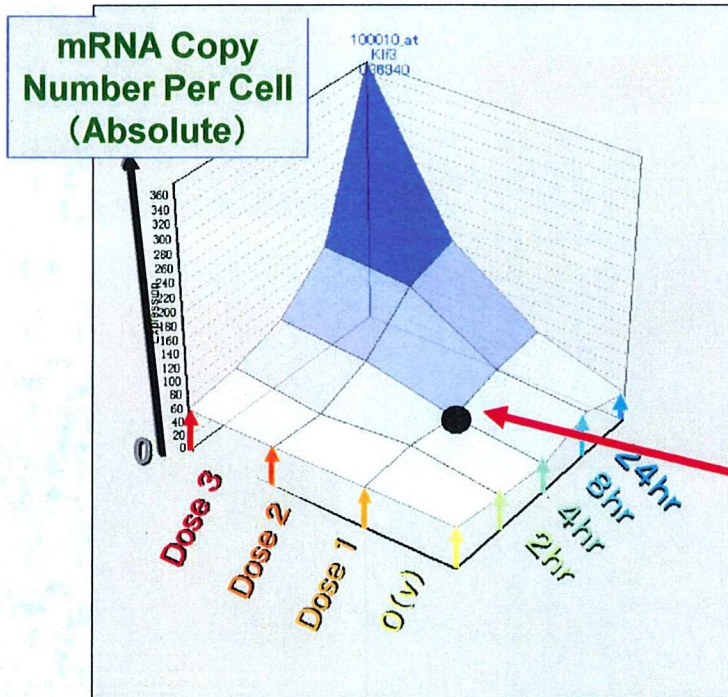
the time- and dose-dependent alternations of gene expression induced in mouse liver (4 time points x 4 dose levels, triplicate, 48 per chemical) are studied on more than 100 chemicals.

Each chemical data is expressed as a 3-D graph (time x dose x copies per cell) made of 45,000 surfaces corresponding to the probe sets of the Affymetrix MOE430 2.0 GeneChip.

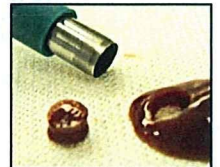
30



# Millefeuille data (MF surface data)

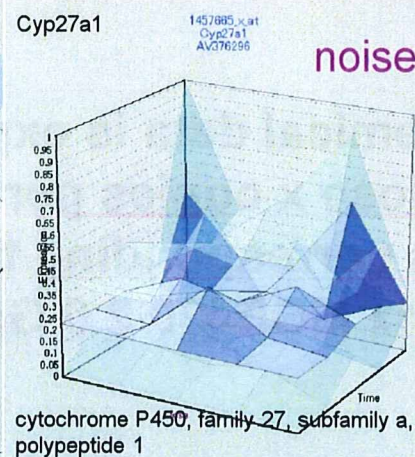
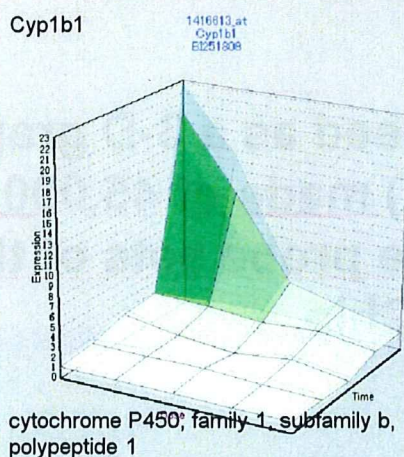


One point:  
 Triplicate  
 (three GeneChips)  
 Mean ± SD  
 4x4x3=48 animals



# Millefeuille data (MF surface data)

Biologist-friendly!  
 Easy to check the data by eyes



One point:  
 (three GeneChips)  
 SD  
 animals



# Percellome Database

- Single exposure orally by gavage
- With dose finding study :
  - little clinical sign
  - little gross abnormalities at 24hour after exposure
- More than 100 chemicals



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## Data base Chemicals : Category classification

Medicine	Isoniazid Cisplatin (Transplatin) Acetaminophen Aspirin Ibuprofen Dexamethasone Omeprazole Phenobarbital Valproic Acid Thalidomide Sodium arsenite (NaAs2HO4) Diethylstilbestrol Tamoxifen Paclitaxel (Taxol) Phenytoin Riampicin PCN AraC	Chemicals related to Chemical Substances Control Law in Japan NSAIDs Teratogen	2,4-dinitrophenol 4-amino-2,6-dichlorophenol Pentachlorophenol 2-Aminomethylpyridine 2-Vinylpyridine 1,2,3-Triazole 1,2,4-Triazole 3-Amino-1H-1,2,4-triazole N-Methylaniline 2-Chloro-4,6-dimethylaniline 1,2-Dichloro-3-nitrobenzene 4-Ethylnitrobenzene
Agricultural chemicals	Paraquat Methoprene Pyriproxyfen Tebufenozide Acephate Carbaryl Warfarin Permethrin Deet	Insect Growth Regulator : IGR	Industrial chemicals Toluene Bromobenzene Carbon tetrachloride Methanol DMSO Tributyltin Bisphenol A MEHP DEHP Fullerene Indigo
Food-derived chemicals	Citric acid Hydroxycitric Acid Forskolin Caffeine Monocrotaline Ethanol Coenzyme Q10 Genistein Genistin Daizein	Diet/Supplement increase in cAMP	DNA demethylating drugs Azacytidine inhalational toxic chemicals Formaldehyde Acetaldehyde
Mutagen	Diethylnitrosamine N-ethyl-N-nitrosourea	Agonists on nuclear receptor	Ethynyl estradiol Testosterone propionate Clofibrate Troglitazone Levothyroxine All trans retinoic acid 9-cis retinoic acid Methoprene acid TCDD TCDF 3-methylcholanthrene

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# Data base Chemicals : Category classification

医薬品 Medicine	<p>Isoniazid Cisplatin (Transplatin) Acetaminophen Aspirin Ibuprofen Dexamethasone Omeprazole Phenobarbital Valproic Acid Thalidomide Sodium arsenite ( NaAs2HO4) Diethylstilbestrol Tamoxifen Paclitaxel (Taxol) Phenytoin Rifampicin PCN AraC</p>	<p>化審法 Chemicals related to Chemical Substances Control Law in Japan</p>	<p>2,4-dinitrophenol 4-amino-2,6-dichlorophenol Pentachlorophenol 2-Aminomethylpyridine 2-Vinylpyridine 1,2,3-Triazole 1,2,4-Triazole 3-Amino-1H-1,2,4-triazole N-Methylaniline 2-Chloro-4,6-dimethylaniline 1,2-Dichloro-3-nitrobenzene 4-Ethynitrobenzene</p>
農業 Agricultural chemicals	<p>Paraquat Methoprene Pyriproxyfen Tebufenozide Acephate Carbaryl Warfarin Permethrin Deet</p>	<p>工業製品 Industrial chemicals</p>	<p>Toluene Bromobenzene Carbon tetrachloride Methanol DMSO Tributyltin Bisphenol A MEHP DEHP Fullerene Indigo</p>
食品 Food-derived chemicals	<p>Citric acid Hydroxycitric Acid Forskolin Caffeine Monocrotaline Coenzyme Q10 Genistein Genistin Daizein</p>	<p>脱メチル化剤 DNA demethylating drugs</p>	<p>Azacytidine</p>
変異原性物質 Mutagen	<p>Diethylnitrosamine N-ethyl-N-nitrosourea</p>	<p>吸入毒性物質 inhalational toxic chemicals</p>	<p>Formaldehyde Acetaldehyde</p>
		<p>核内受容体作動性物質 Agonists on nuclear receptor</p>	<p>Ethynyl estradiol Testosterone propionate Clotibrate Troglitazone Levothyroxine All trans-retinoic acid 9-cis-retinoic acid Methoprene acid TCDD TCDF 3-methylcholanthrene</p>

Analog

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TT0012 Thalidomide	TT0017 Paracetamol derivative	TT0014 2,4-dichlorophenol	TT0015 2-Aminomethylpyridine	TT0016 N-methylaniline	TT0019 2-Vinylpyridine	TT0020 2,4-dinitrophenol	TT0021 4-amino-2,6-dichlorophenol	TT0022 Pentachlorophenol	TT0023 2-Aminomethylpyridine	TT0024 2-Vinylpyridine	TT0025 1,2,3-Triazole	TT0026 1,2,4-Triazole	TT0027 3-Amino-1H-1,2,4-triazole	TT0028 N-Methylaniline	TT0029 2-Chloro-4,6-dimethylaniline	TT0030 1,2-Dichloro-3-nitrobenzene	TT0031 4-Ethynitrobenzene
TT0032 1H-1,2,3-triazole	TT0033 1H-1,2,4-triazole	TT0034 2-Aminomethylpyridine	TT0035 N-methylaniline	TT0036 2-Amino-1H-1,2,4-triazole	TT0037 2-Vinylpyridine	TT0038 2,4-dinitrophenol	TT0039 4-amino-2,6-dichlorophenol	TT0040 Pentachlorophenol	TT0041 2-Aminomethylpyridine	TT0042 2-Vinylpyridine	TT0043 1,2,3-Triazole	TT0044 1,2,4-Triazole	TT0045 3-Amino-1H-1,2,4-triazole	TT0046 N-Methylaniline	TT0047 2-Chloro-4,6-dimethylaniline	TT0048 1,2-Dichloro-3-nitrobenzene	TT0049 4-Ethynitrobenzene
TT0050 Acetaminophen	TT0051 Acetaminophen	TT0052 Aspirin	TT0053 Ibuprofen	TT0054 Dexamethasone	TT0055 Omeprazole	TT0056 Phenobarbital	TT0057 Valproic acid	TT0058 Thalidomide	TT0059 Sodium arsenite	TT0060 Diethylstilbestrol	TT0061 Tamoxifen	TT0062 Paclitaxel	TT0063 Phenytoin	TT0064 Rifampicin	TT0065 PCN	TT0066 AraC	
TT0067 Paraquat	TT0068 Methoprene	TT0069 Pyriproxyfen	TT0070 Tebufenozide	TT0071 Acephate	TT0072 Carbaryl	TT0073 Warfarin	TT0074 Permethrin	TT0075 Deet									
TT0076 Citric acid	TT0077 Hydroxycitric Acid	TT0078 Forskolin	TT0079 Caffeine	TT0080 Monocrotaline	TT0081 Coenzyme Q10	TT0082 Genistein	TT0083 Genistin	TT0084 Daizein									
TT0085 Diethylnitrosamine	TT0086 N-ethyl-N-nitrosourea																

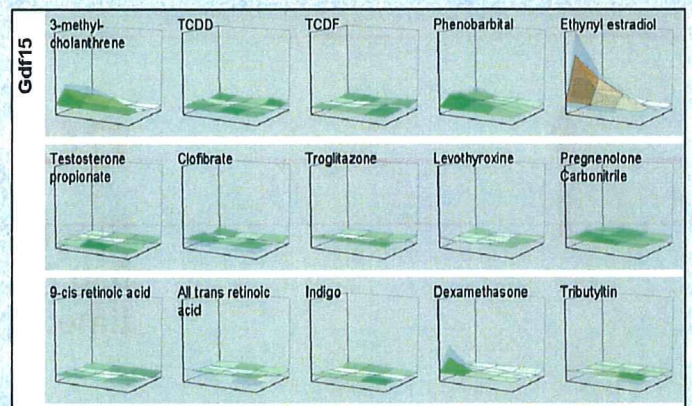
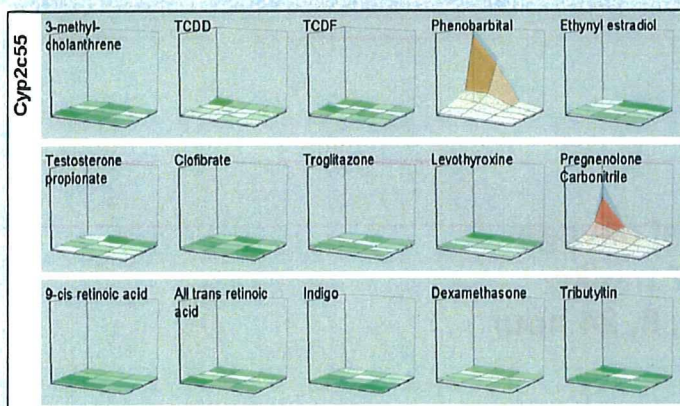
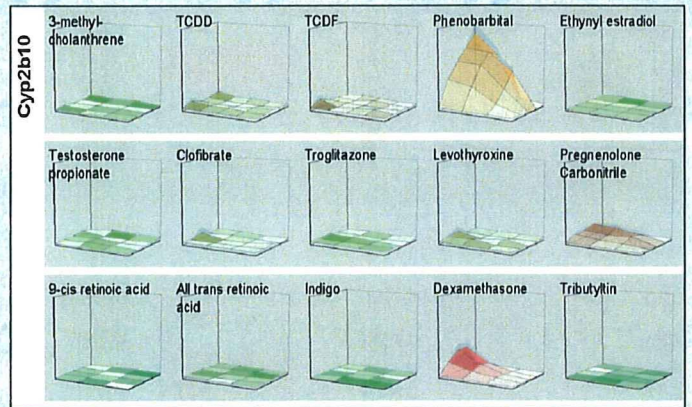
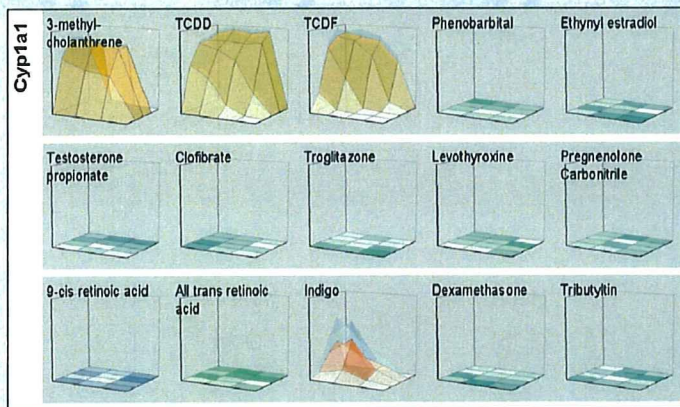
36





Cyp2a12

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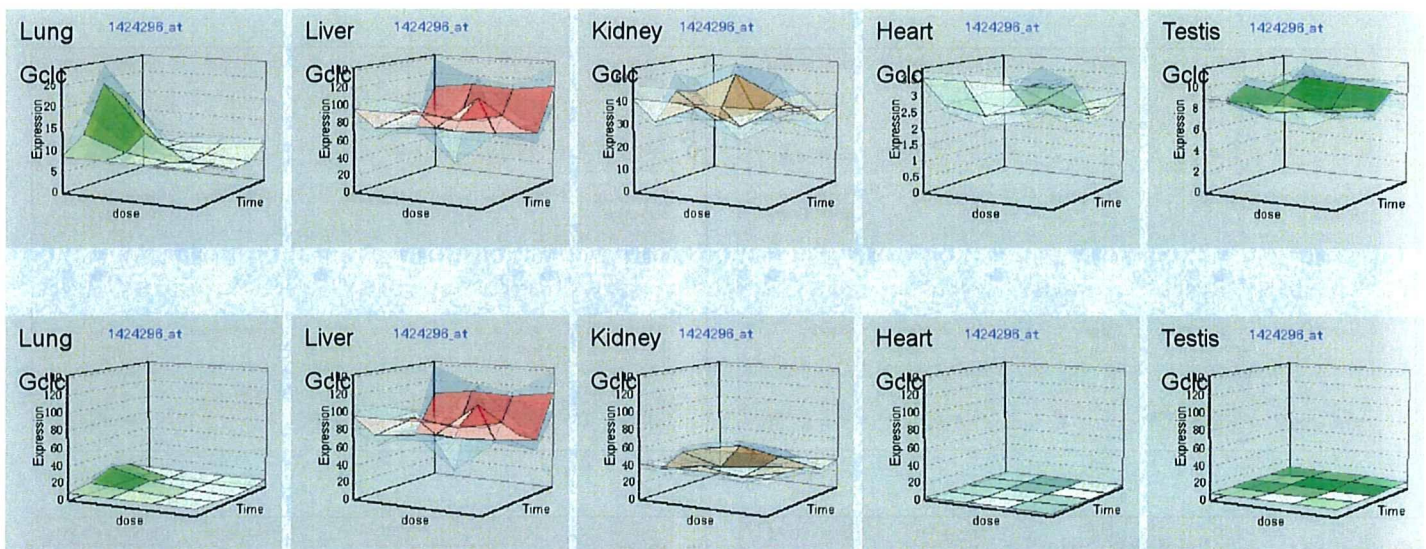
38



# Examples of direct data comparison among different organs



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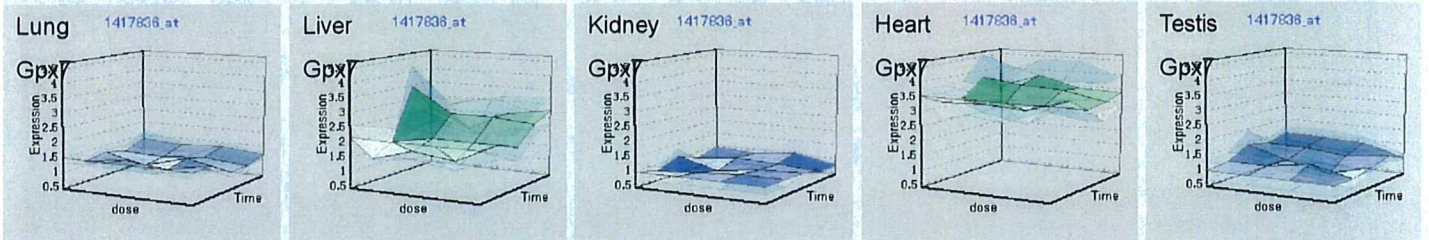
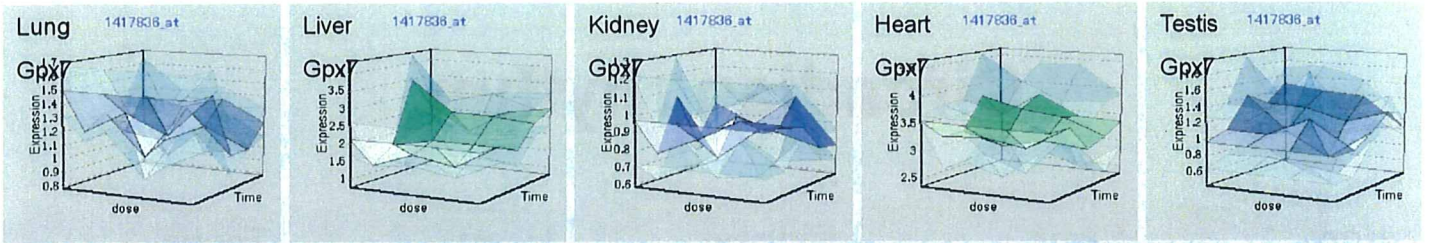
Single oral gavage  
0, 0.7, 2, 7 mg/kg  
time: 2, 4, 8, 24 hour

Gclc

glutamate-cysteine ligase, catalytic subunit

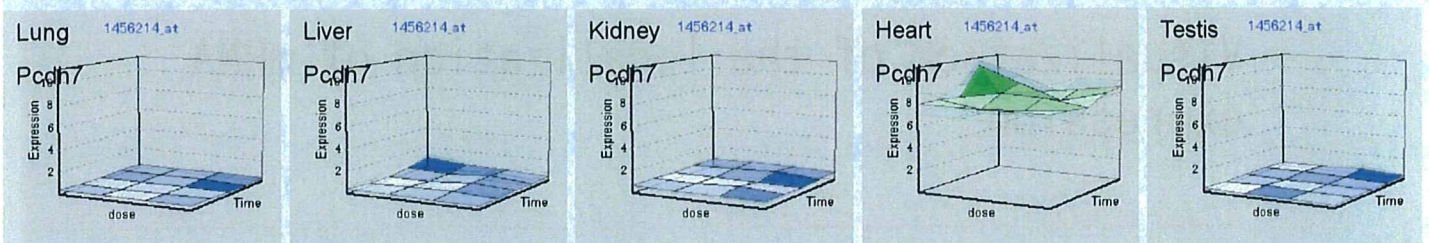
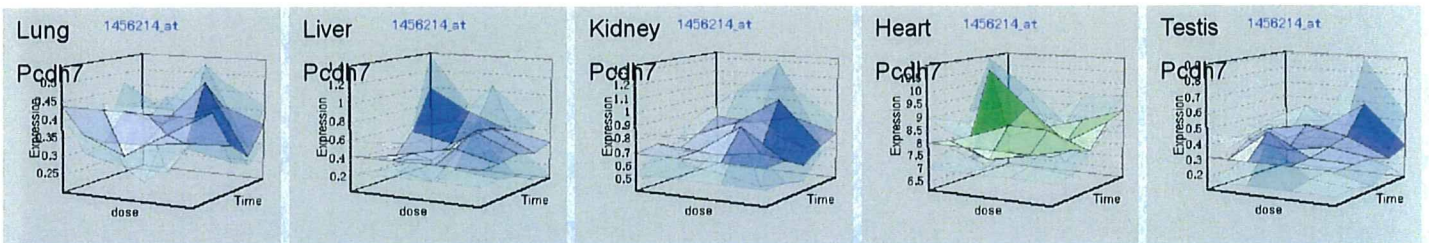
40





### Gpx7

glutathione peroxidase 7

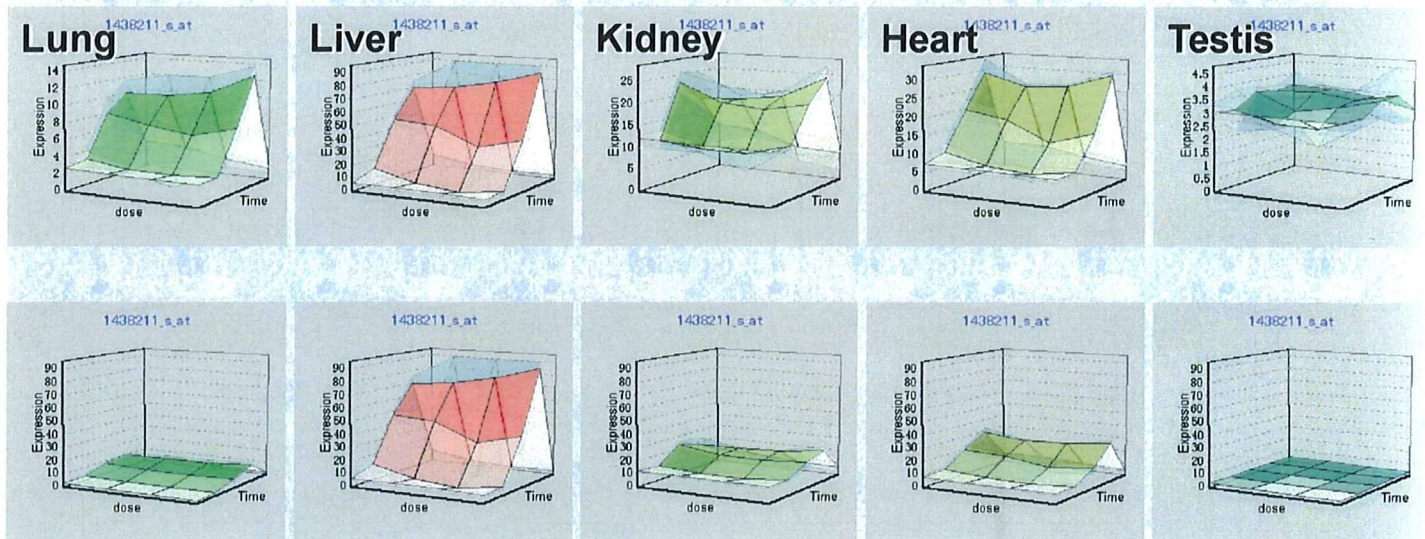


### Pcdh7

protocadherin 7



# Circadian rhythm



Not only the light cycle but also administration-time and sampling-time are critical

## Dbp

D site albumin promoter binding protein

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- In situ hybridization
  - Visualization of the localization of mRNA expression



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