Percellome toxicogenomics project as the 3R-toxicology and the foundation of *in vitro*- and *in silico*-toxicology

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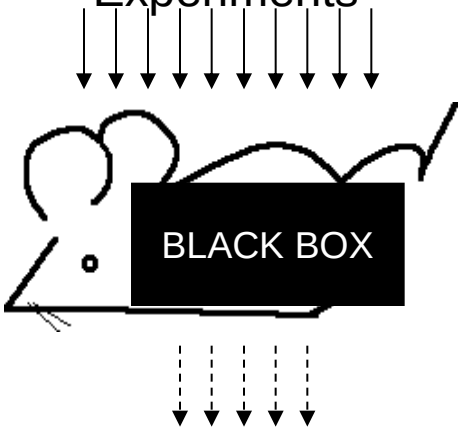
http://www.nihs.go.jp/tox/TttgPublished.htm

12~13th century drawing 鳥獣人物戯画より

Do we Use the Magic Number "SAFETY FACTOR" ***for ever*** ?

To avoid 2nd "Thalidomide Story"

Chemical Exposure Experiments



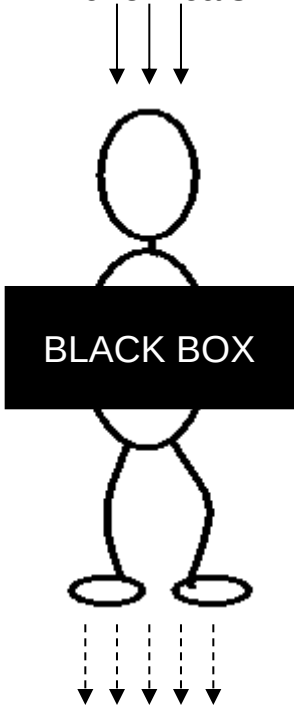
Phenotypes (toxicity)

Basic assumption = similar response may occur in humans and experimental animals

Safety Factors

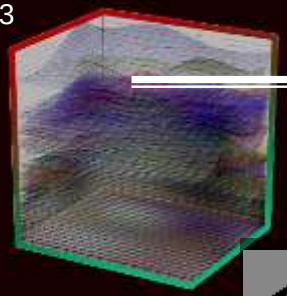
SNPs-like factors were incorporated as safety factors based on experience.

Accidental exposure to chemicals



Phenotypes (toxicity)

Modernization of Risk Assessment



Chemicals

BLACK BOX

Gene KO models

Uncertainty factor

Chemicals

SNPs

BLACK BOX

Some gene function will directly connect the mechanism of toxicity
Or
will make the Uncertainty Factors more certain

phenotype

Phenotype-independent

Responsible gene

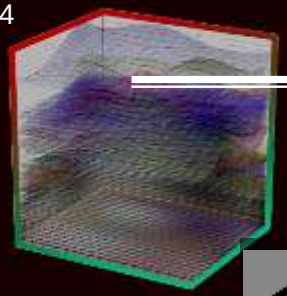
phenotype

Phenotype-independent

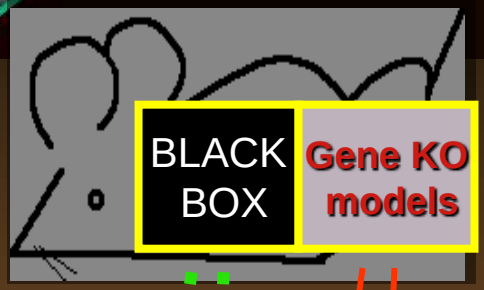
Responsible gene

Ultimate goal: virtual mouse, virtual human *(cf. Star Trek 24th century !)*

Modernization of Risk Assessment

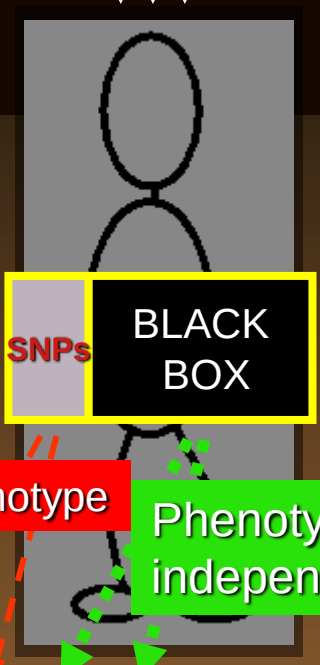


Chemicals



Uncertainty factor

Chemicals



Some gene function will directly connect the mechanism of toxicity
Or
will make the Uncertainty Factors more certain

Phenotype-independent

phenotype

phenotype

Phenotype-independent

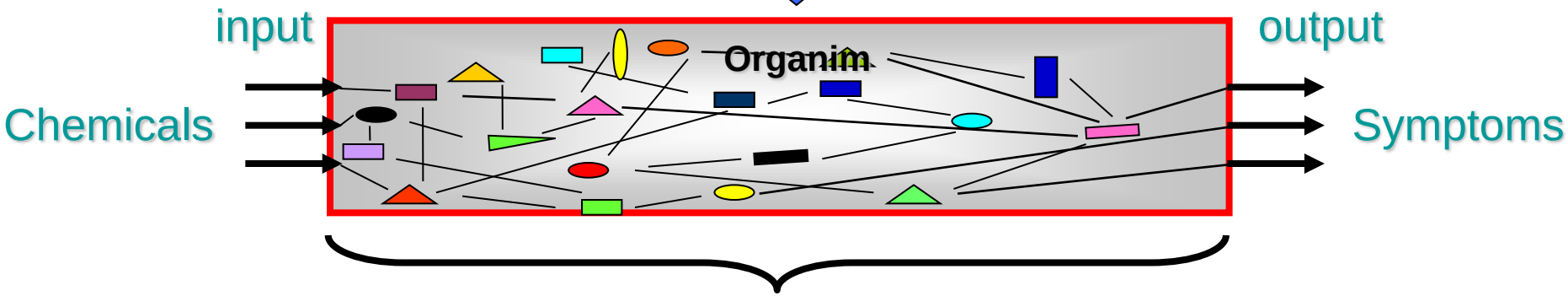
Responsible gene

Responsible gene

Ultimate goal: virtual mouse, virtual human *(cf. Star Trek 24th century !)*

Why monitor all genes comprehensively

Mechanism-based modernization



Development of Network Database by *All genes*
(not only "VIP" genes)

↓

Not all networks accompany phenotype

Data accumulation needs data standardization
= absolute copy numbers of mRNA per one cell (average)
= Percellome method

BMC Genomics

BioMed Central

Methodology article

Open Access

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

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* Corresponding author †Equal contributors

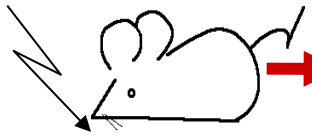
Open Access : ***BMC Genomics. 2006 Mar 29;7(1):64***

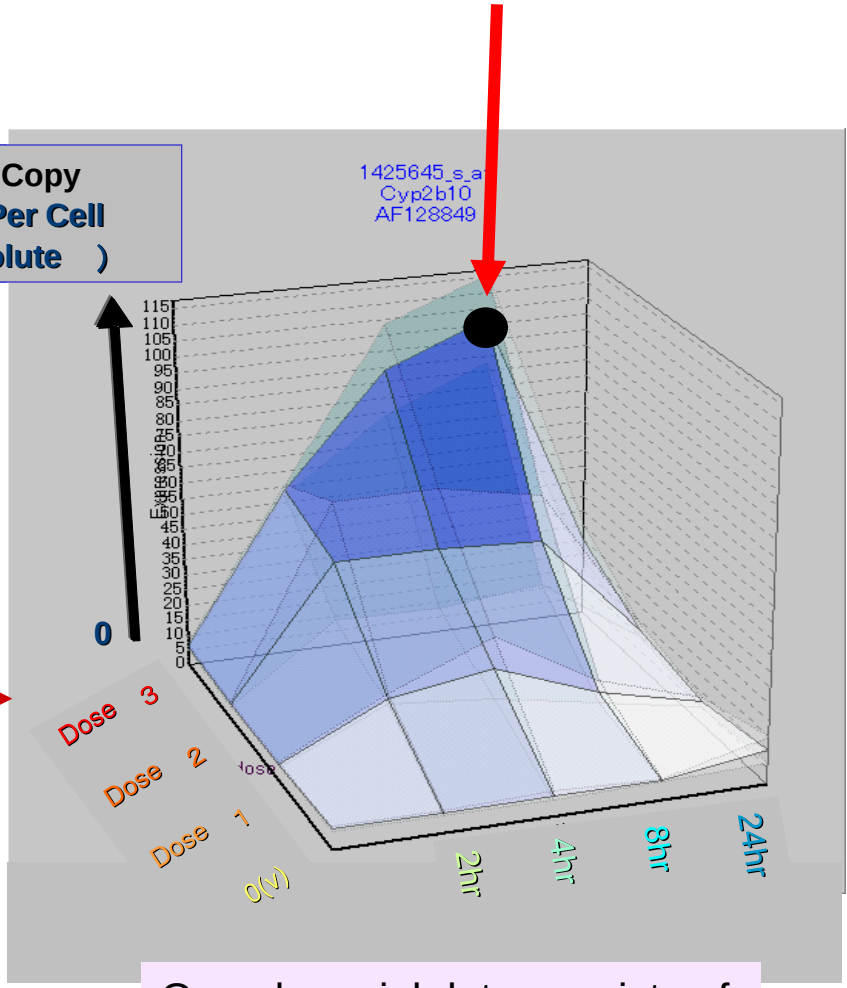
Standard Protocol of Percellome Project

Mean or mode of n=3
And ±1sd

Oral, Inhalation

Chemicals





Per One Chemical,
4 time points (2hr, 4hr, 8hr, 24hr)
4 dose levels (0, 1, 2, 3)

16 groups n=3
48 mice
Liver (Lung, Kidney, brain, etc)

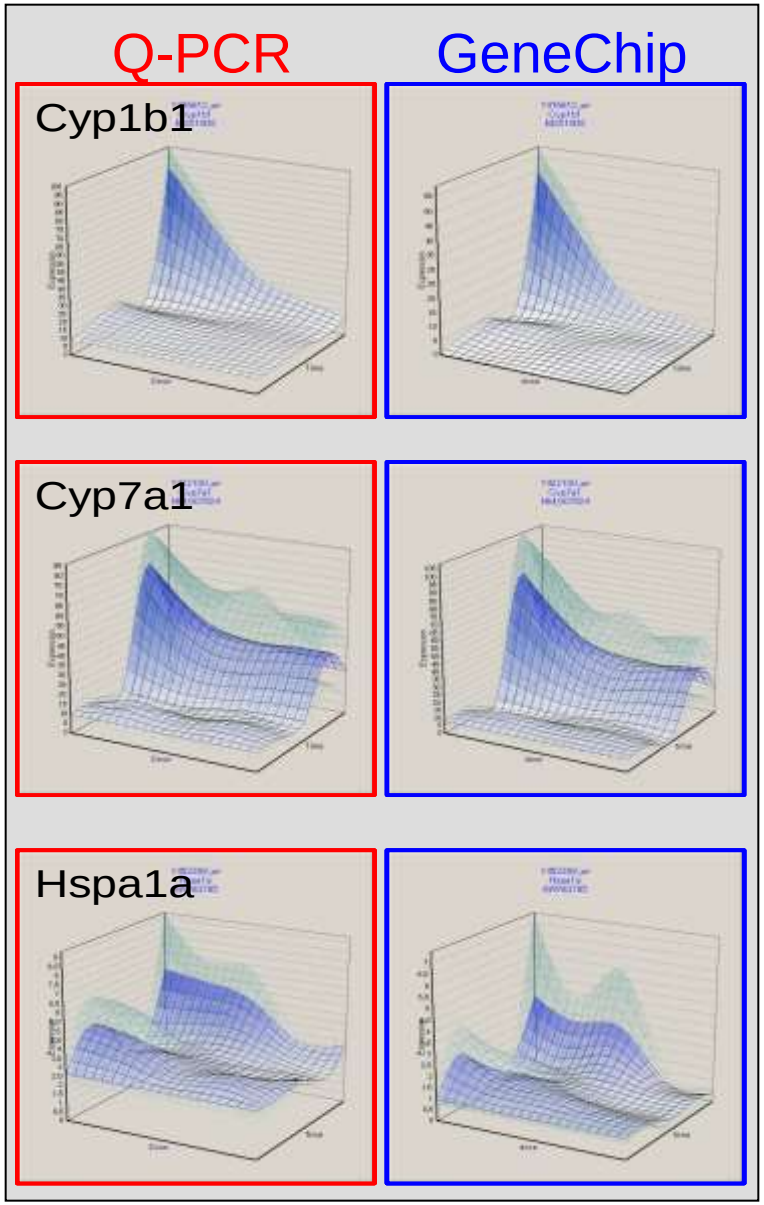
Affymetrix GeneChip MOE430

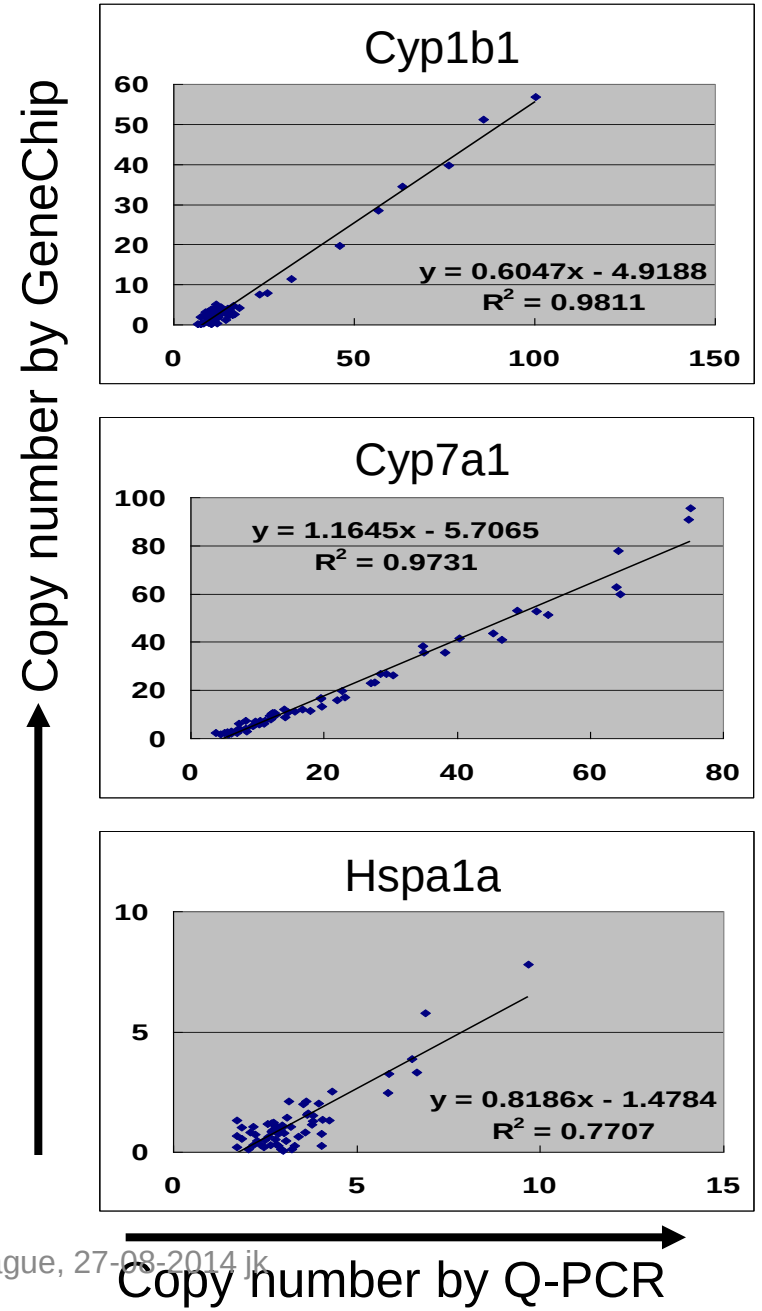
45,000 probe set data

One chemcial data consists of 45,000 layers of 3D surface

Percellome Q-PCR versus GeneChip

TCDD /// 5x4 n=3, 60 sample /// GSC(5)+19 primer pair set1

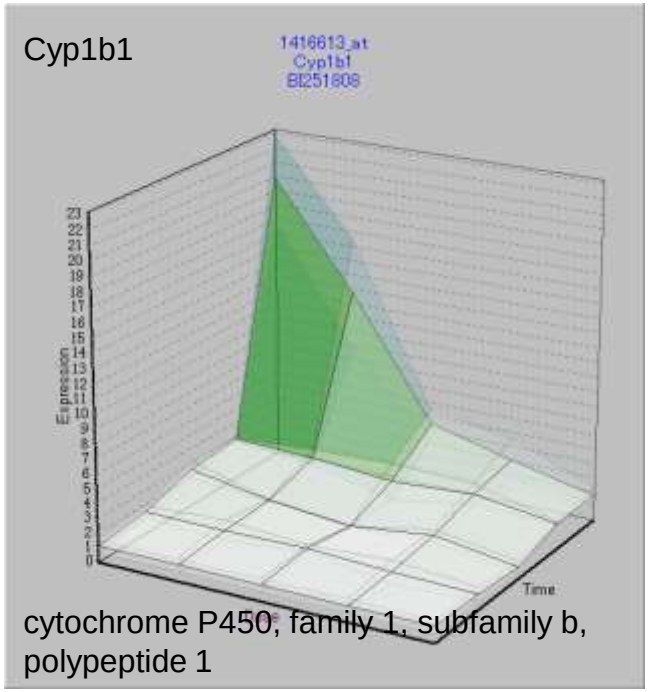




- Features of Percellome data
  - Profile-independent
    - Time course data
    - Different organs/ tissues
    - Different subspecies/ species
  - Absolute value (scalable from Zero)
    - Easy to add, subtract, multiply, divide
    - Direct comparison among samples, and studies

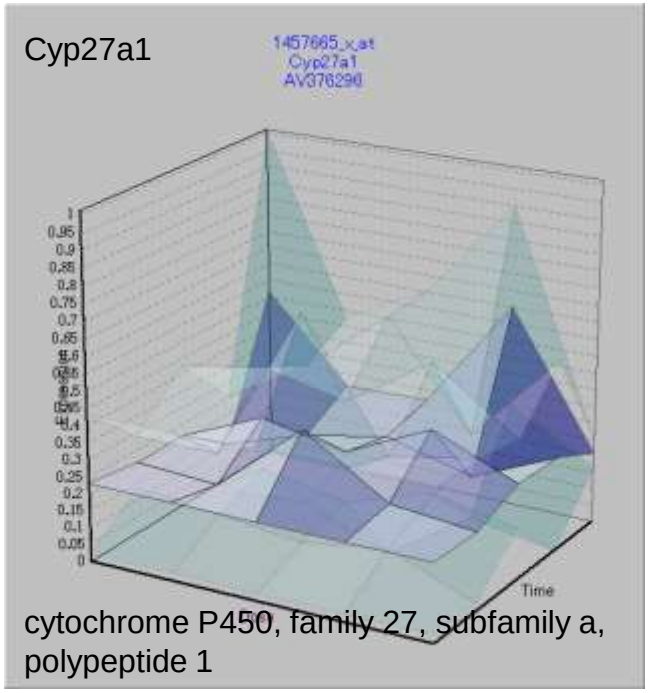
Percellome Surface data is Biologist-friendly! Easy to check by your eyes

Cyp1b1



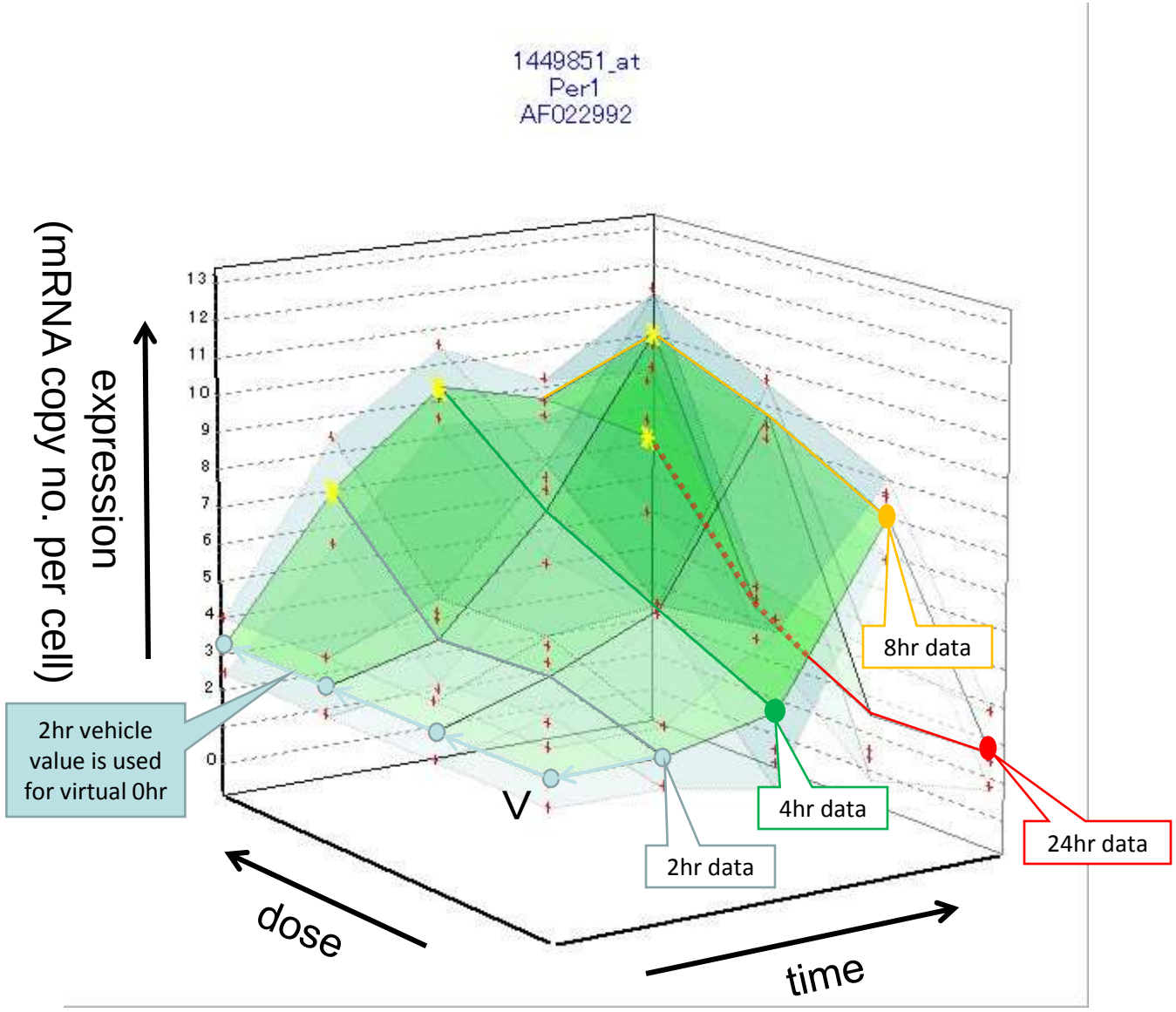
cytochrome P450, family 1, subfamily b, polypeptide 1

Cyp27a1



cytochrome P450, family 27, subfamily a, polypeptide 1

Figure 1


1449851_at
Per1
AF022992
expression
(mRNA copy no. per cell)
13
12
11
10
9
8
7
6
5
4
3
2
0
2hr vehicle value is used for virtual 0hr
V
2hr data
4hr data
8hr data
24hr data
dose
time

Acephate
Acetaldehyde
Acetaminophen
Agaritine
Alpha lipoic acid
3-Amino-1H-1,2,4-triazole
4-amino-2,6-dichlorophenol
2-Aminomethylpyridine
AraC
Aspirin
Azacytidine
Benzene
Bisphenol A
Bromobenzene
Caffeine
Carbaryl
Carbon tetrachloride
2-Chloro-4,6-dimethylaniline
Cisplatin
Citric acid-calcium salt
Clofibrate
Coenzyme Q10
Curcumin
Daizein
Deet
DEHP
Dexamethasone
Dibutyltin dichloride
1,2-Dichloro-3-nitrobenzene
Diethylnitrosamine (C57BL/6)
Diethylstilbestrol
Digitoxin
2,4-dinitrophenol
DMSO
Domoic acid
Estragole
Ethanol
4-Ethylnitrobenzene
Ethynyl estradiol
FK506
Formalin
Forskolin
Fullerene
Genistein
Genistin
Glycyrrhizin2K
Hydroxycitric Acid
Hydroxycitric Acid
Ibuprofen (dl-p-isobutylhydratropic acid)
Indigo
Isoniazid
Kanamycin monosulfate
Levothyroxine
Maltol
MEHP
Menthyl Valerate
Methanol
Methoprene
Methoprene acid
Methyl dihydro jasmonate
3-methylcholanthrene
Monocrotaline
Nerolidol
N-ethyl-N-nitrosourea
N-Methylaniline
Omeprazole
Paclitaxel (Taxol)
Paraquat dichloride
Pentachlorophenol
Permethrin
Phenobarbital sodium
Phenytoin
Phytol
5-Pregnen-3beta-ol-20-one-16alpha-carbonitrile
Pyriproxyfen
9-cis retinoic acid
All trans retinoic acid
Rifampicin
Sodium Arsenite
Sodium Dehydroacetate
Tamoxifen
TCDD(2,3,7,8-Tetrachlorodibenzo-p-Dioxin)
TCDF(2,3,7,8-Tetrachlorodibenzofuran)
Tebufenozide
Testosterone propionate
Thalidomide
Toluene
Transplatin
1,2,3-Triazole
1,2,4-Triazole
Tributyltin chloride
Troglitazone
Valproic acid sodium salt
Verbenone
2-Vinylpyridine
Warfarin
青色1号 Blue No.1
青色2号 Blue No.2
赤色40号 Red No.40
赤色226号 Red No.226

Acephate
Acetaldehyde
Acetaminophen
Agaritine
Alpha lipoic acid
3-Amino-1H-1,2,4-triazole
4-amino-2,6-dichlorophenol
2-Aminomethylpyridine
DMSO
Domoic acid
Estragole
Ethanol
4-Ethylnitrobenzene
Ethynyl estradiol
FK506
Formalin
Paclitaxel (Taxol)
Paraquat dichloride
Pentachlorophenol
Permethrin
Phenobarbital sodium
Phenytoin
Phytol
5-Pregnen-3beta-ol-20-one-16alpha-carbonitrile

About 100 kinds of Chemicals
More than 300 data (48 GeneChip data per Organ)

oxin)
)

Daizein
Deet
DEHP
Dexamethasone
Dibutyltin dichloride
1,2-Dichloro-3-nitrobenzene
Diethylnitrosamine (C57BL/6)
Diethylstilbestrol
Digitoxin
2,4-dinitrophenol
Methanol
Methoprene
Methoprene acid
Methyl dihydro jasmonate
3-methylcholanthrene
Monocrotaline
Nerolidol
N-ethyl-N-nitrosourea
N-Methylaniline
Omeprazole
1,2,4-Triazole
Tributyltin chloride
Troglitazone
Valproic acid sodium salt
Verbenone
2-Vinylpyridine
Warfarin
青色1号 Blue No.1
青色2号 Blue No.2
赤色40号 Red No.40
赤色226号 Red No.226

Dose selection for Percellome studies

- Top dose:
  - Default setting
    - dose that does not induce macroscopic and microscopic changes in 24 hours after single dose.
  - Hormones or hormone-like chemicals (nuclear receptor ligands)
    - dose that gives near maximum response (ED70~80) (referring to binding assay or reporter gene assay data)
- Middle and Low dose:
  - $\sqrt{10}$ ratio (10, 3, 1 or 20, 7, 2)

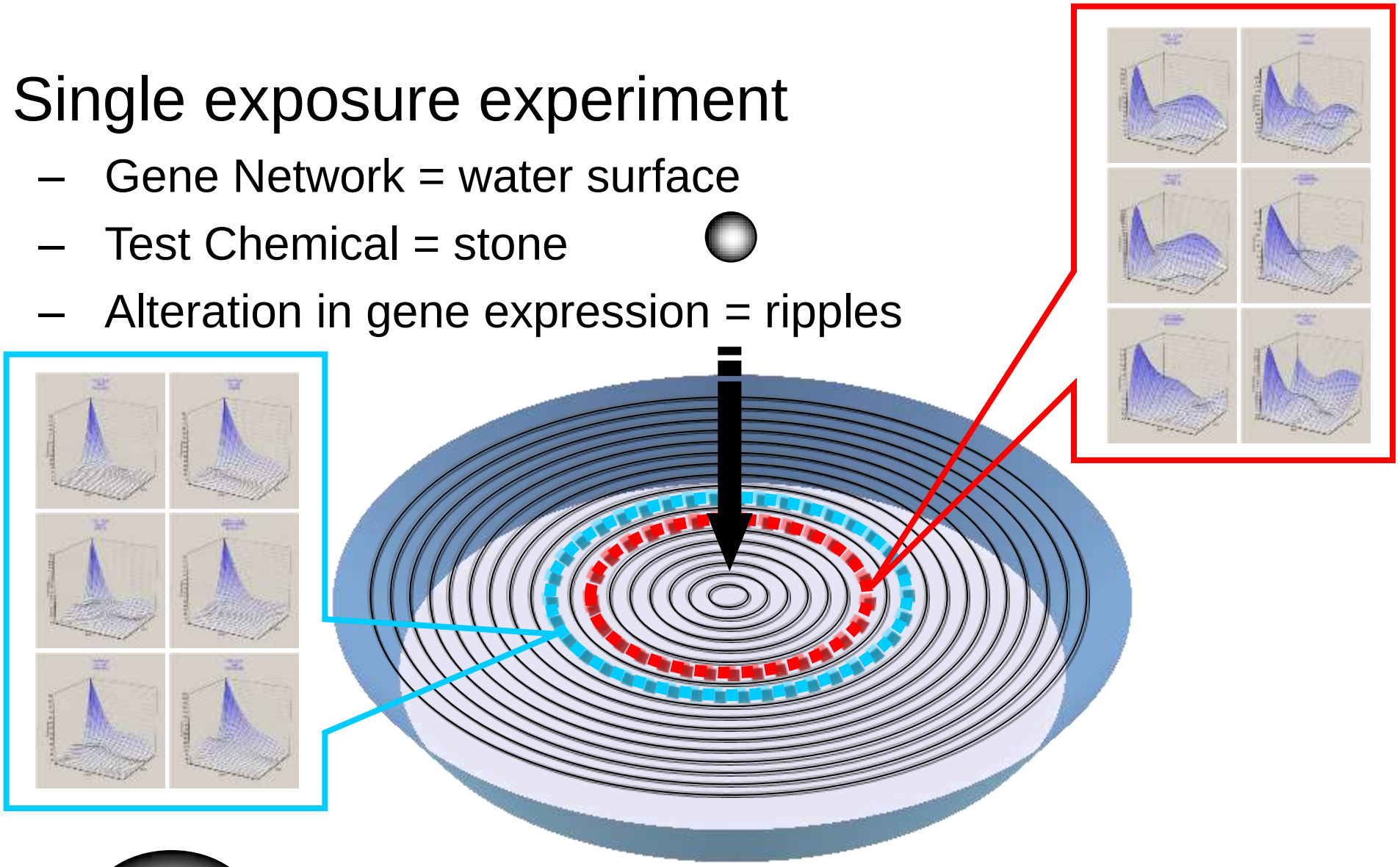
Dose selection

In short, Signal dose!

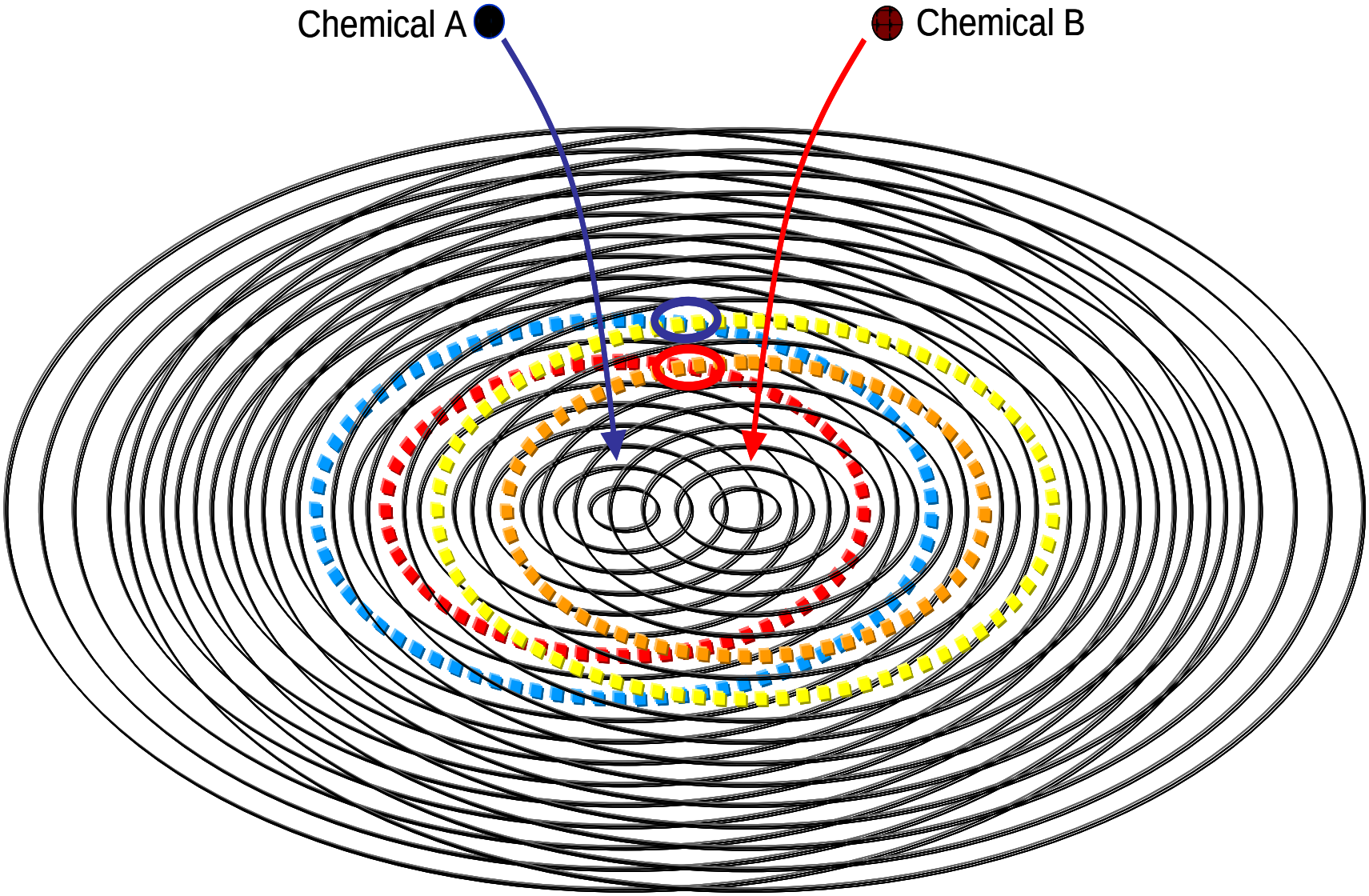
- Top dose:
  - Default setting
    - dose that does not induce macroscopic and microscopic changes in 24 hours after single dose.
  - Hormones or hormone-like chemicals (nuclear receptor ligands)
    - dose that gives near maximum response (ED70~80) (referring to binding assay or reporter gene assay data)
- Middle and Low dose:
  - $\sqrt{10}$ ratio (10, 3, 1 or 20, 7, 2)

- Percellome data analysis strategy
  - Comprehensive
  - Unsupervised

- Single exposure experiment
  - Gene Network = water surface
  - Test Chemical = stone
  - Alteration in gene expression = ripples

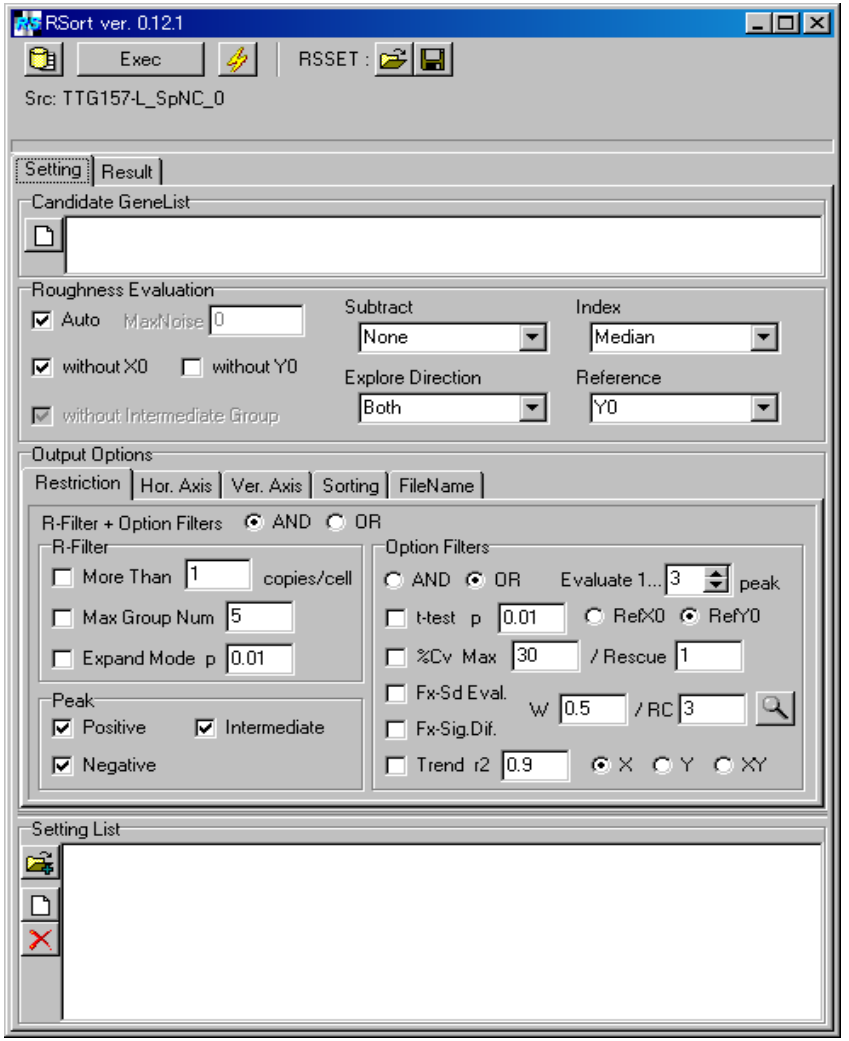


Chemical → Protein etc.→ Gene → Protein → Gene → Protein →・・・・・


Chemical A
Chemical B

RSort Program by Ken-ichi Aisaki

- 3-dimensional surfaces are Sorted by its Roughness of the shape, detecting the position of + and – peaks.

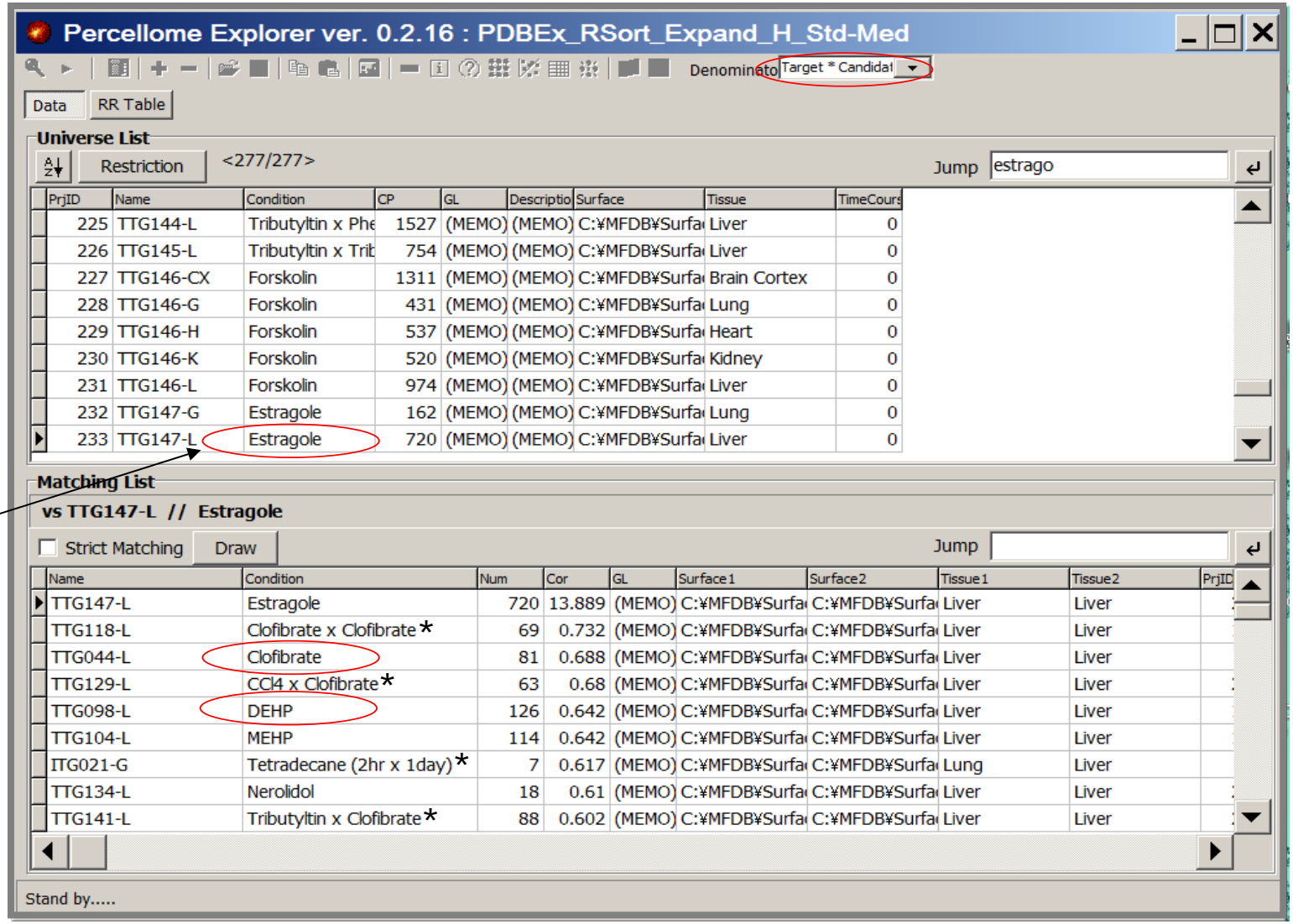


PercellomeExplorer Program by K Aisaki

Upper window:
RSort -selected Percellome data sets regardless of organ

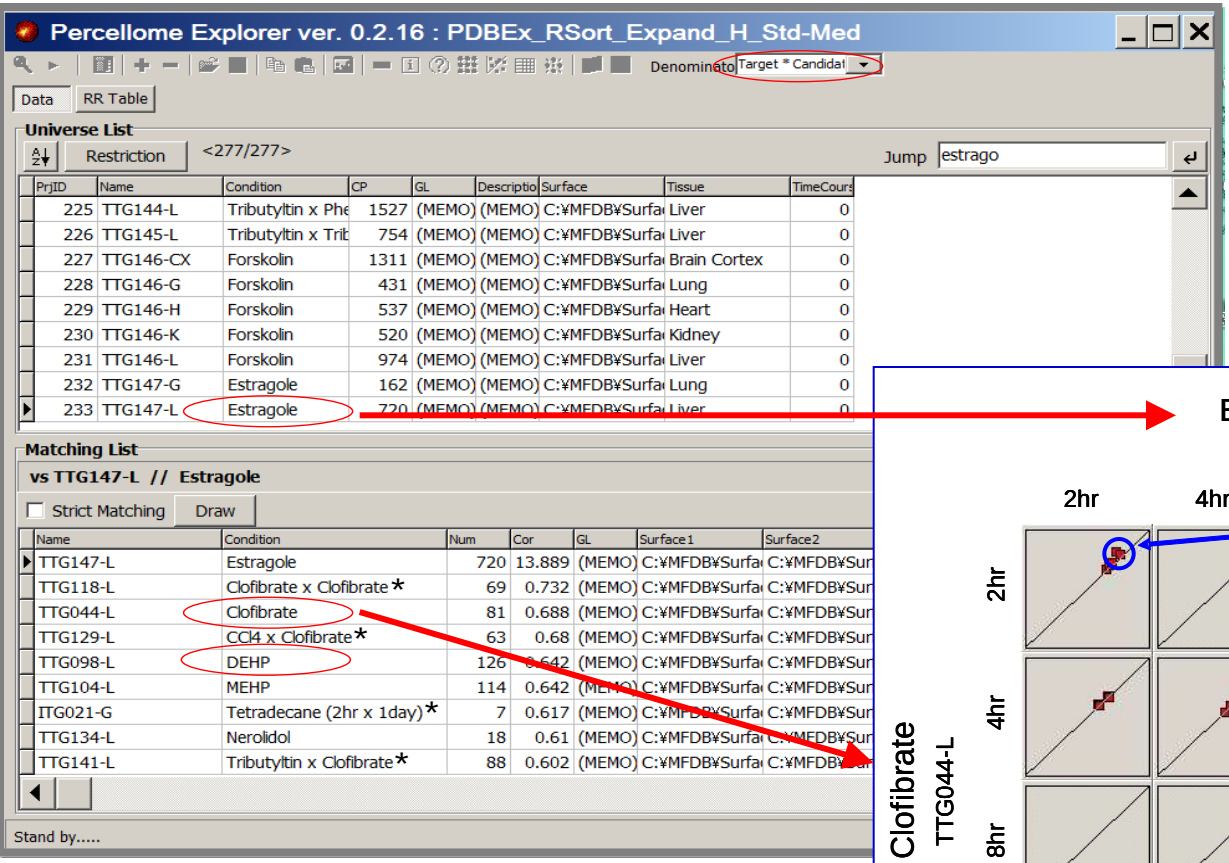
Click the data of interest

Lower window:
Data sharing more PSs are listed



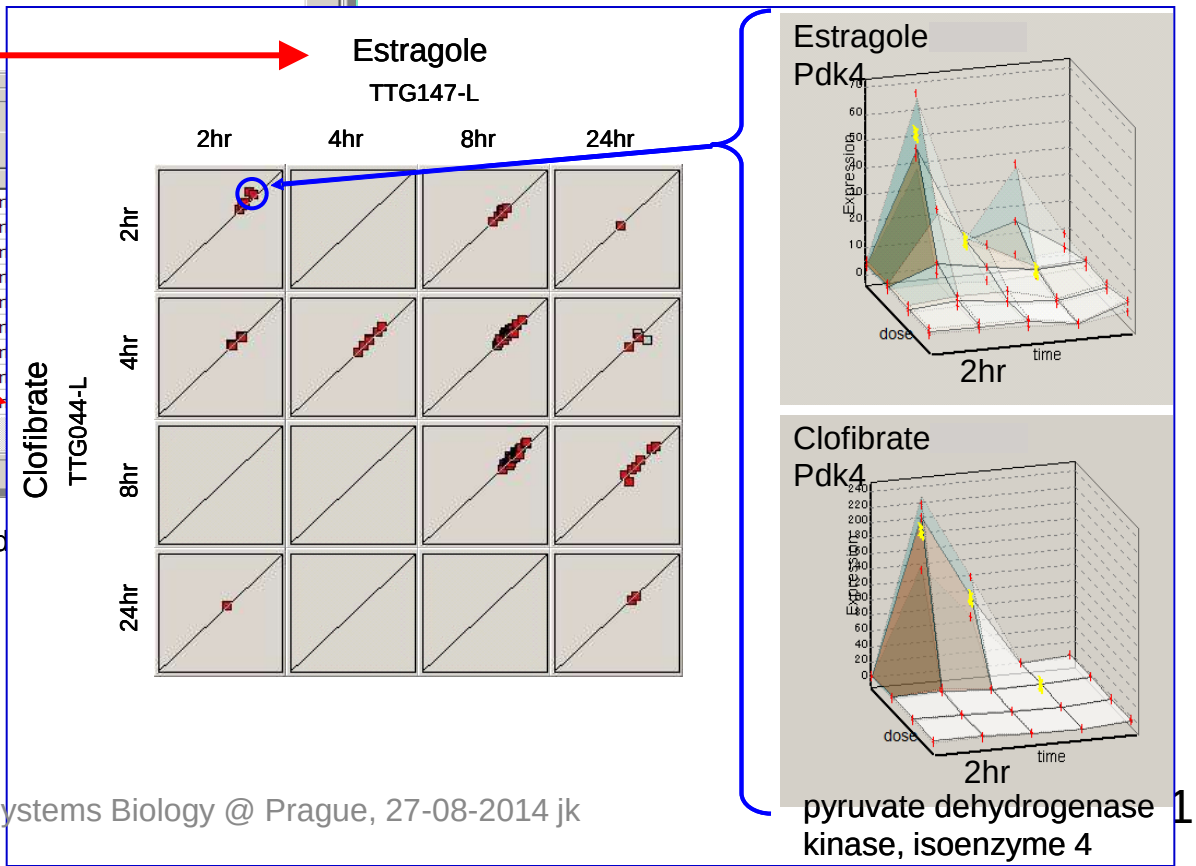
*:Performed by different protocols

PercellomeExplorer Program by K Aisaki



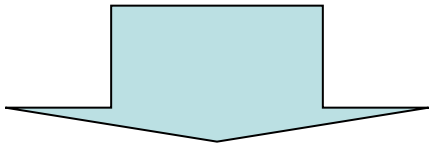
*:Performed

Any pair can be selected to show PSs peaked at vairous time and draw the 3D surface graphs

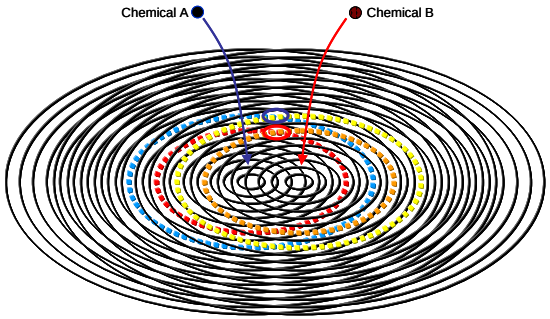


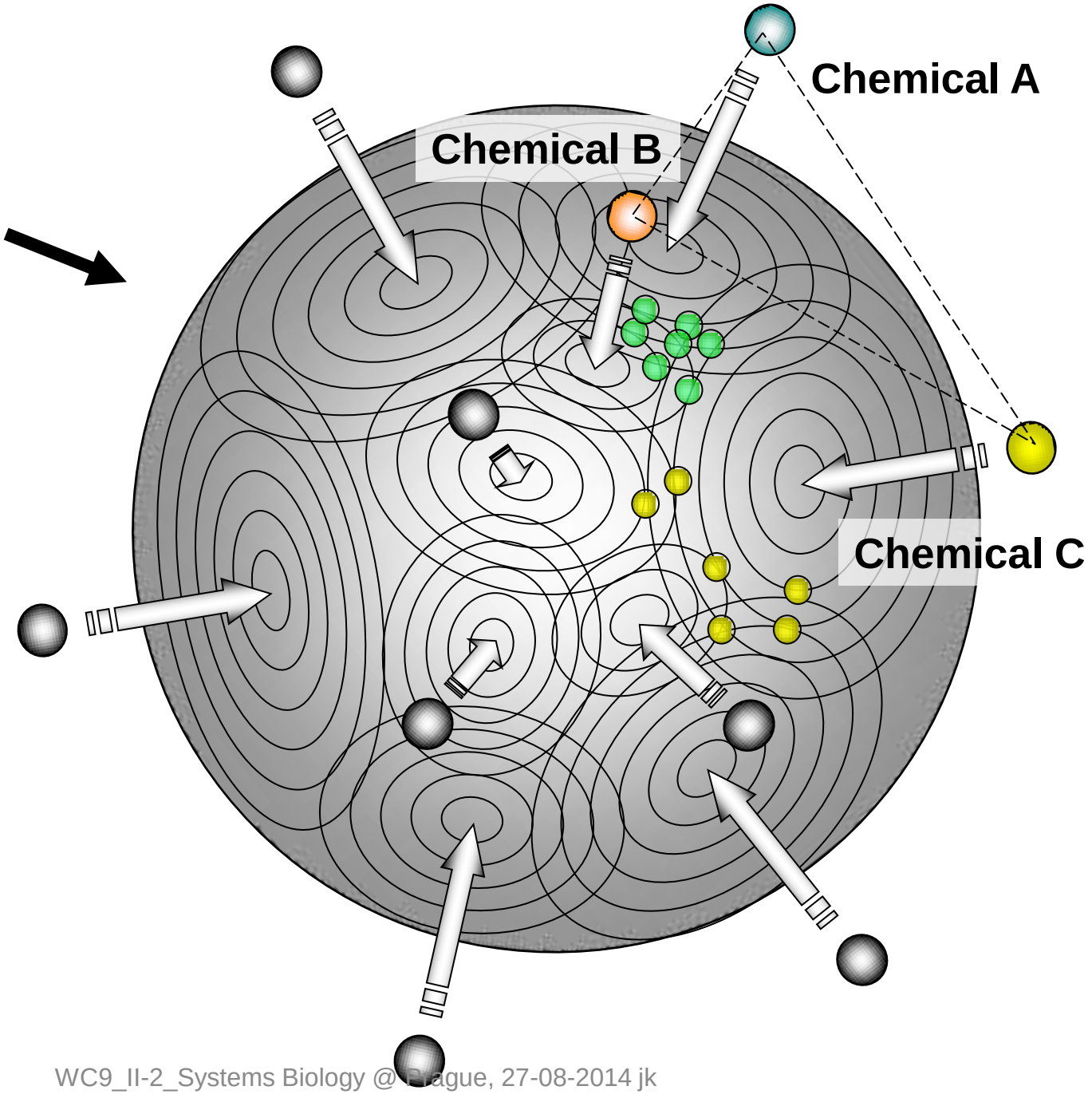
pyruvate dehydrogenase kinase, isoenzyme 4

- Percellome data analysis strategy
  - Comprehensive
  - Unsupervised

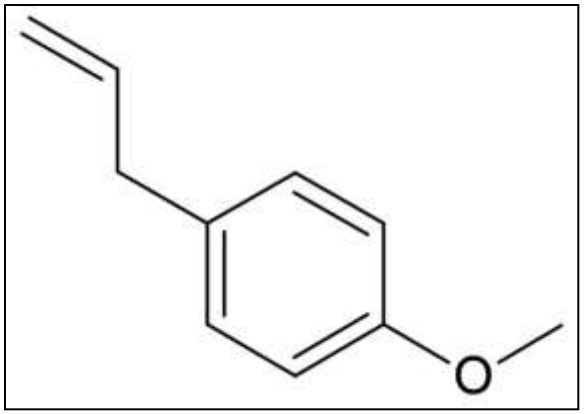


- Comprehensive Network Drawing


Chemical A
Chemical B


Chemical A
Chemical B
Chemical C

Flavor "estragole"



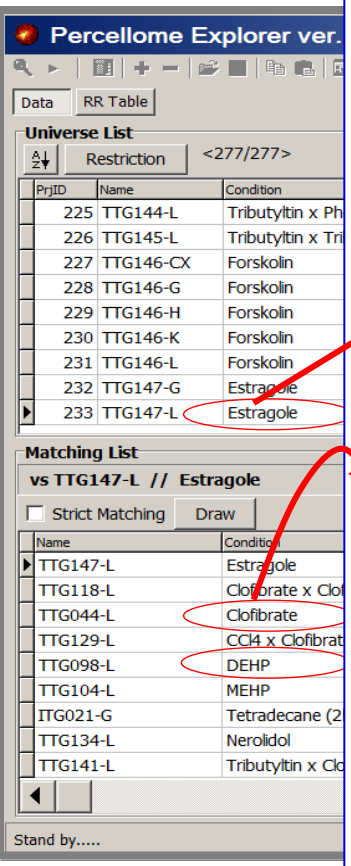
(1-allyl-4-methoxybenzene)
(CAS No.: 140-67-0)

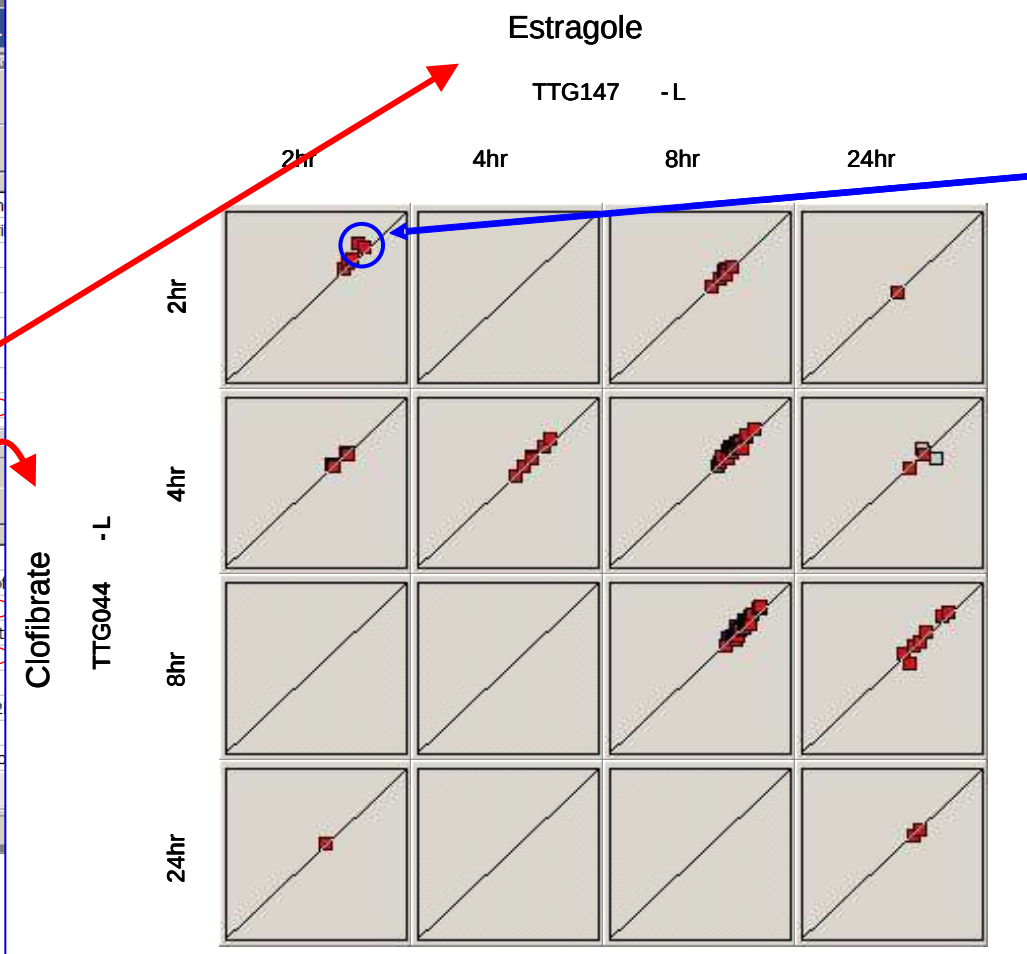
a major component of tarragon, essential oils of basil, and others.

reported to induce hepatomas in rodents at higher dosages (JECFA)

Kanno *et al.,* Application of Percellome Toxicogenomics to Food Safety
In: Issues in Toxicology No.11. Hormone-Disruptive Chemical Contaminants in Food.
Eds. Ingemar Pongratz and Vikstroem Bergander
Royal Society of Chemistry, London, pp. 184-198 (2012)

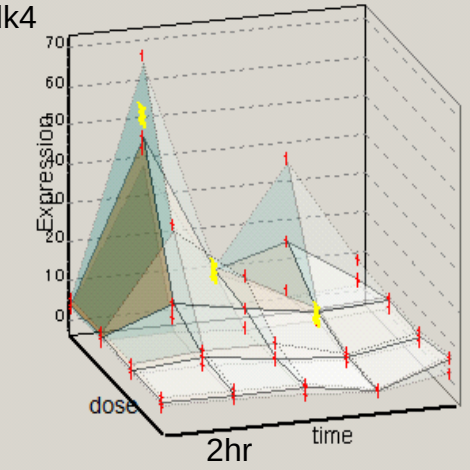
PercellomeExplorer Program by K Aisaki





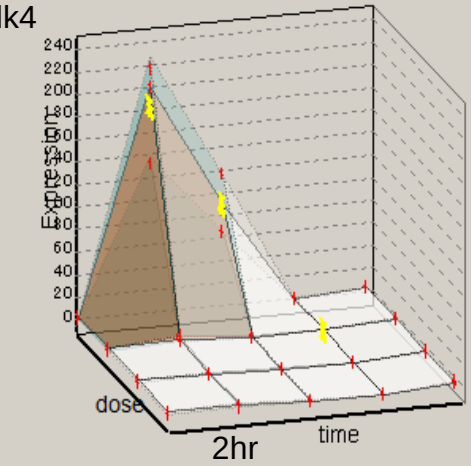
Estragole

Pdk4

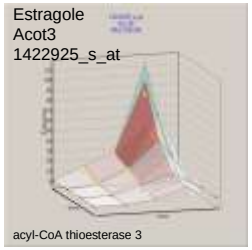


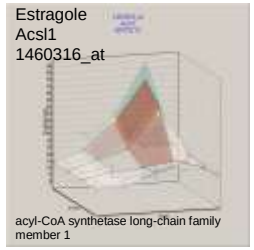
Clofibrate

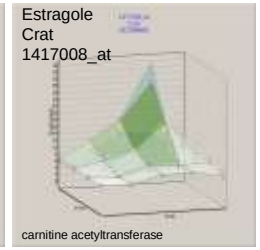
Pdk4

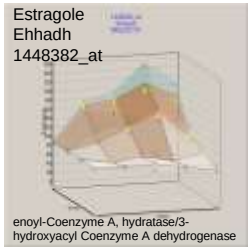


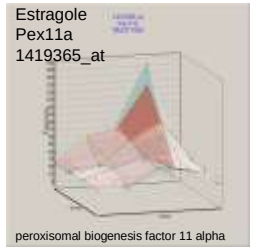
pyruvate dehydrogenase kinase, isoenzyme 4

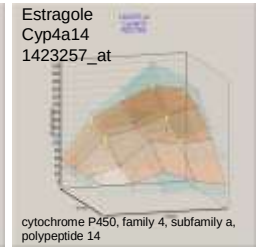

Estragole
Acot3
1422925_s_at
acyl-CoA thioesterase 3

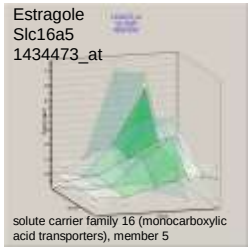

Estragole
Acsl1
1460316_at
acyl-CoA synthetase long-chain family member 1

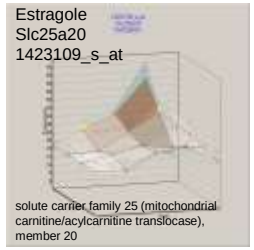

Estragole
Crat
1417008_at
carnitine acetyltransferase

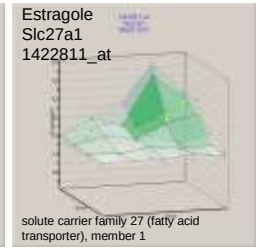

Estragole
Ehhadh
1448382_at
enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase


Estragole
Pex11a
1419365_at
peroxisomal biogenesis factor 11 alpha

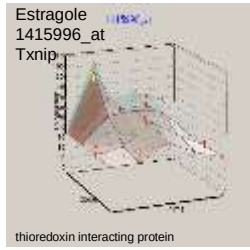

Estragole
Cyp4a14
1423257_at
cytochrome P450, family 4, subfamily a, polypeptide 14

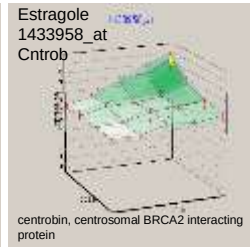

Estragole
Slc16a5
1434473_at
solute carrier family 16 (monocarboxylic acid transporters), member 5

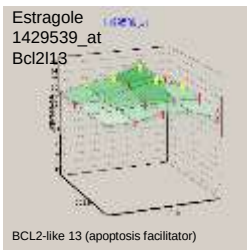

Estragole
Slc25a20
1423109_s_at
solute carrier family 25 (mitochondrial carnitine/acylcarnitine translocase), member 20

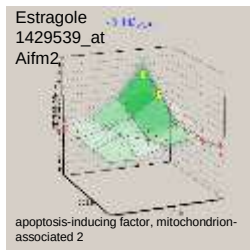

Estragole
Slc27a1
1422811_at
solute carrier family 27 (fatty acid transporter), member 1

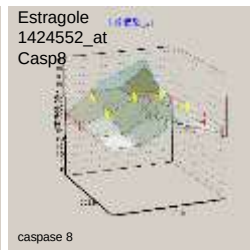

Estragole
1416592_at
Glrx
glutaredoxin

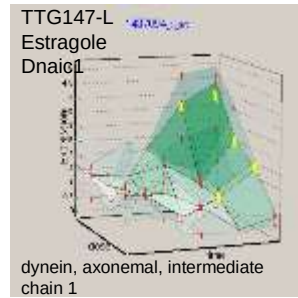

Estragole
1415996_at
Txnip
thioredoxin interacting protein

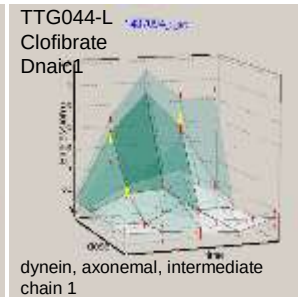

Estragole
1433958_at
Cntrob
centrobin, centrosomal BRCA2 interacting protein

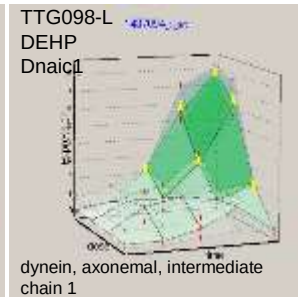

Estragole
1429539_at
Bcl2l13
BCL2-like 13 (apoptosis facilitator)

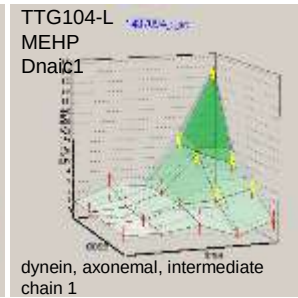

Estragole
1429539_at
Aifm2
apoptosis-inducing factor, mitochondrion-associated 2

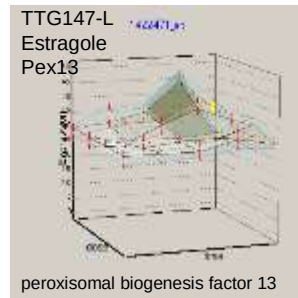

Estragole
1424552_at
Casp8
caspase 8

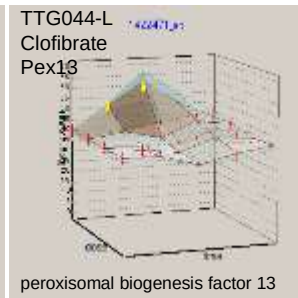

TTG147-L
Estragole
Dnaic1
dynein, axonemal, intermediate chain 1

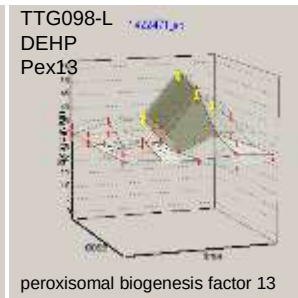

TTG044-L
Clofibrate
Dnaic1
dynein, axonemal, intermediate chain 1

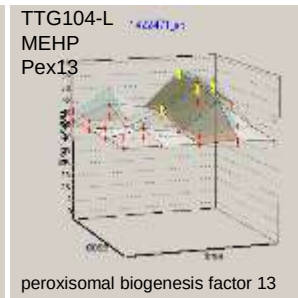

TTG098-L
DEHP
Dnaic1
dynein, axonemal, intermediate chain 1

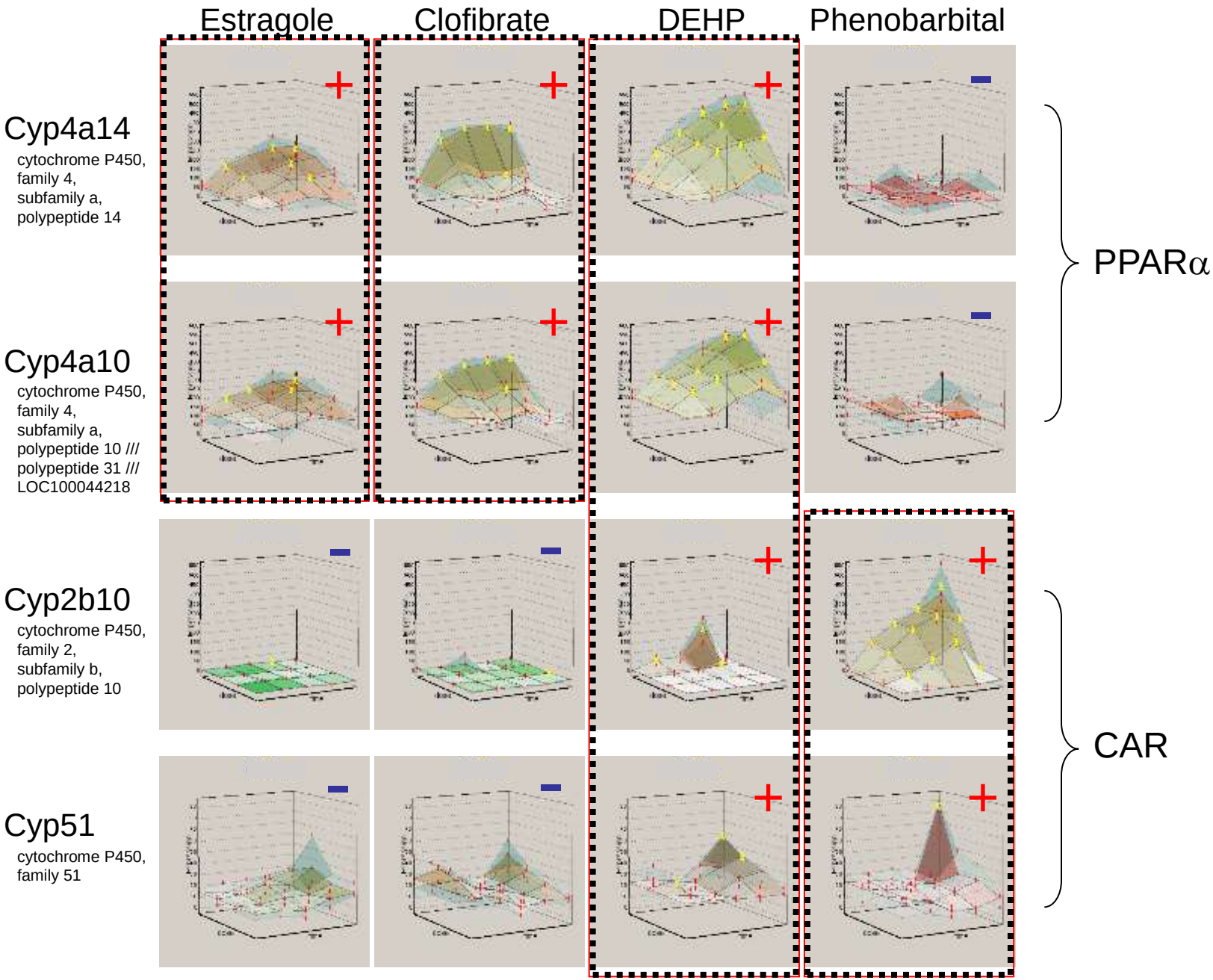

TTG104-L
MEHP
Dnaic1
dynein, axonemal, intermediate chain 1


TTG147-L
Estragole
Pex13
peroxisomal biogenesis factor 13

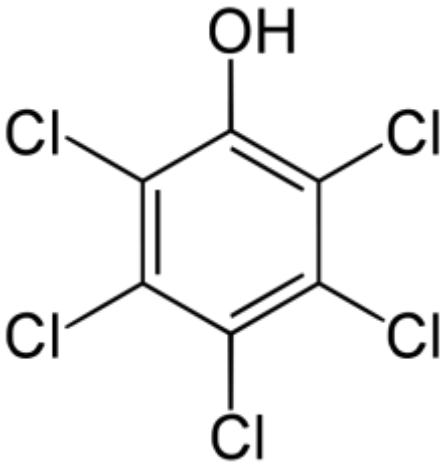

TTG044-L
Clofibrate
Pex13
peroxisomal biogenesis factor 13


TTG098-L
DEHP
Pex13
peroxisomal biogenesis factor 13


TTG104-L
MEHP
Pex13
peroxisomal biogenesis factor 13


Estragole
Clofibrate
DEHP
Phenobarbital
Cyp4a14
cytochrome P450, family 4, subfamily a, polypeptide 14
Cyp4a10
cytochrome P450, family 4, subfamily a, polypeptide 10 /// polypeptide 31 /// LOC100044218
Cyp2b10
cytochrome P450, family 2, subfamily b, polypeptide 10
Cyp51
cytochrome P450, family 51
PPARα
CAR

Pentachlorophenol (PCP)



Used as herbicide, insecticide, fungicide, disinfectant, and other preservative purposes

Acute symptoms:

Morphological changes as

Damages to liver, kidney, hematology, lung, neural system, immune, GI tract

Functional changes as

Hyperthermia, profuse sweating, nausea, uncoordinated movements, etc.

Chronic symptoms:

Liver, kidney, neural

Liver tumor induction

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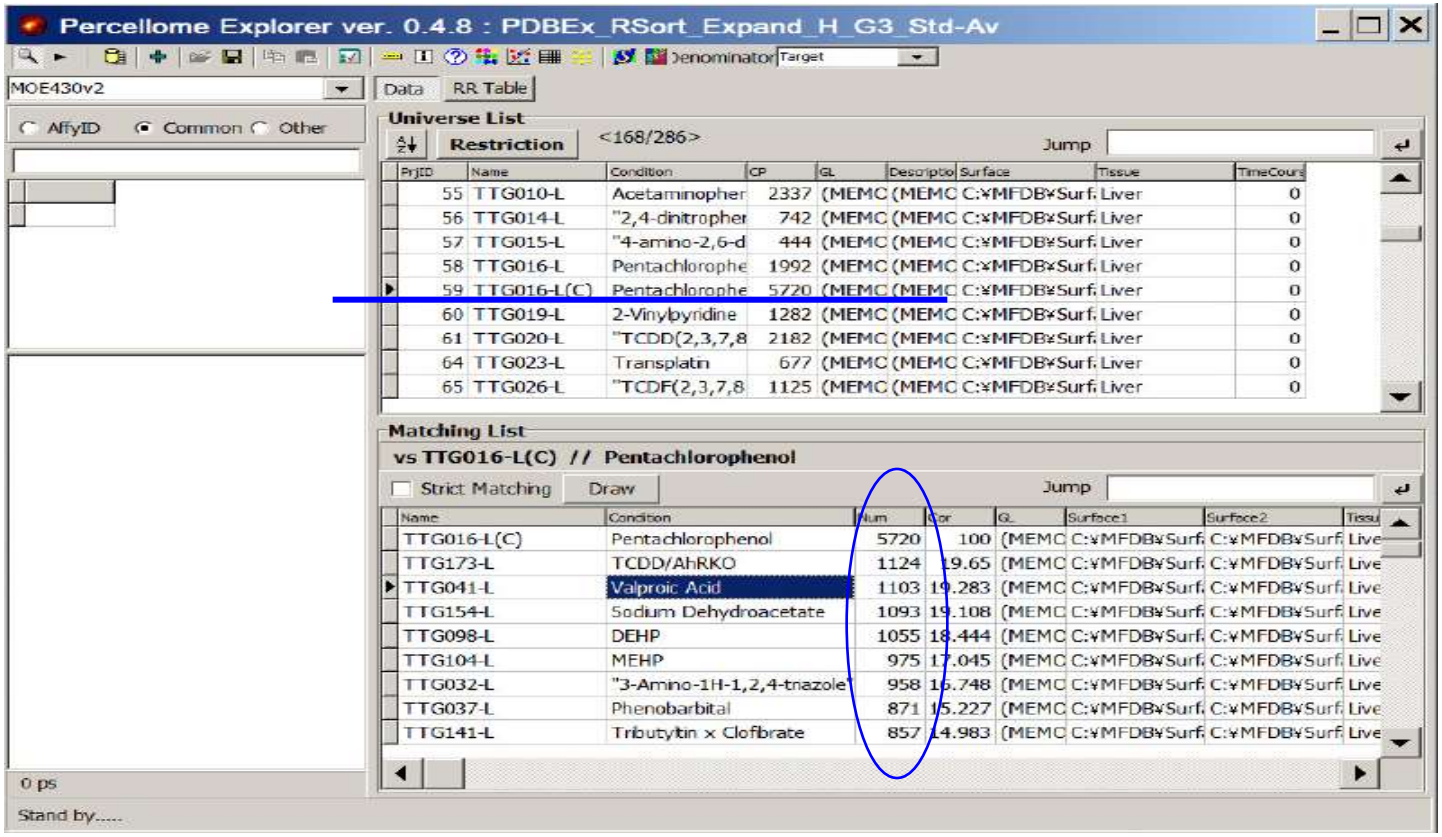
Figure 2



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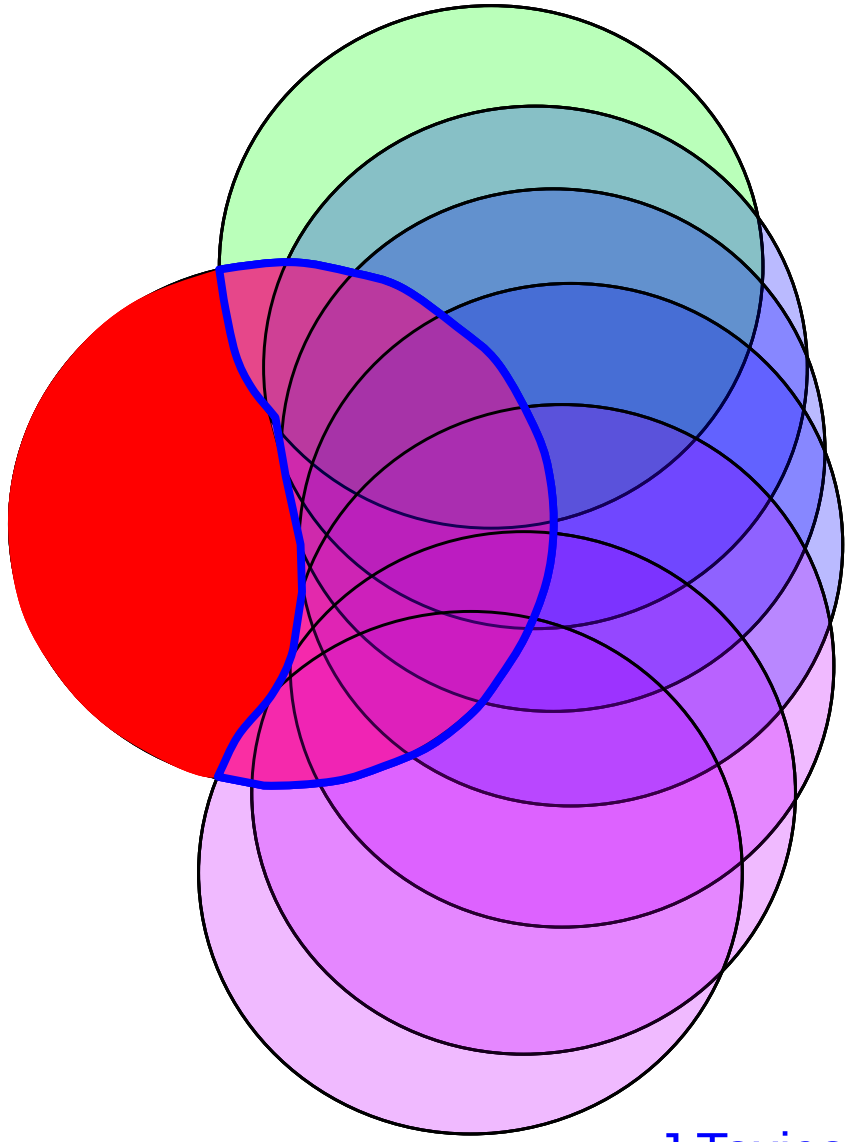
PCP



Other chemicals
in Percellome DB

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2hr 4hr 8hr 24hr

2hr			4hr			8hr			24hr		
Percellome No.	Treatment	PS	Percellome No.	Treatment	PS	Percellome No.	Treatment	PS	Percellome No.	Treatment	PS
TTG016-L(C)	Pentachlorophenol	98	TTG016-L(C)	Pentachlorophenol	55	TTG016-L(C)	Pentachlorophenol	127	TTG016-L(C)	Pentachlorophenol	1192
TTG154-L	Sodium Dehydroacetate	51	TTG104-L	MEHP	21	TTG098-L	DEHP	15	TTG098-L	DEHP	258
TTG109-L	Acephate	24	TTG098-L	DEHP	16	TTG041-L	Valproic Acid	14	TTG032-L	3-Amino-1H-1,2,4-triazole	212
TTG059-L	Caffeine	19	TTG037-L	Phenobarbital	14	TTG104-L	MEHP	14	TTG104-L	MEHP	177
TTG041-L	Valproic Acid	18	TTG032-L	3-Amino-1H-1,2,4-triazole	12	TTG109-L	Acephate	13	TTG037-L	Phenobarbital	160
TTG062-L(C)	Dexamethasone	18	TTG144-L	Tributyltin x Phenobarbital	12	TTG160-L	5-fluorouracil	10	TTG041-L	Valproic Acid	109
TTG098-L	DEHP	17	TTG150-L	Valproic acid sodium salt x Thalidomide	8	TTG154-L	Sodium Dehydroacetate	9	TTG157-L	Valproic acid sodium salt	103
TTG019-L	2-Vinylpyridine	15	TTG141-L	Tributyltin x Clofibrate	8	TTG141-L	Tributyltin x Clofibrate	8	TTG031-L	2-Chloro-4,6-dimethylaniline	94
TTG104-L	MEHP	12	TTG074-L	Bromobenzene	8	TTG031-L	2-Chloro-4,6-dimethylaniline	8	TTG154-L	Sodium Dehydroacetate	77
TTG165-L	Chlorpyrifos	12	TTG151-L	Valproic acid sodium salt x Valproic acid sodium salt	7	TTG032-L	3-Amino-1H-1,2,4-triazole	8	TTG162-L	Sesame seed oil unsaponified matter	71
TTG034-L	4-Ethylnitrobenzene	12	TTG031-L	2-Chloro-4,6-dimethylaniline	7	TTG146-L	Forskolin	6	TTG044-L	Clofibrate	69
TTG166-L	Carbaryl	10	TTG044-L	Clofibrate	6	TTG062-L(C)	Dexamethasone	6	TTG074-L	Bromobenzene	47
TTG031-L	2-Chloro-4,6-dimethylaniline	10	TTG162-L	Sesame seed oil unsaponified matter	5	TTG054-L	Diethylnitrosamine (C57BL/6)	5	TTG109-L	Acephate	17
TTG141-L	Tributyltin x Clofibrate	9	TTG173-L	TCDD/AhRKO	0	TTG132-L	Curcumin	3	TTG160-L	5-fluorouracil	13
TTG032-L	3-Amino-1H-1,2,4-triazole	9				TTG136-L	Phytol	2			
TTG027-L	1,2,3-Triazole	9				TTG096-L	Omeprazole	0			
TTG160-L	5-fluorouracil	4									
	Sum Set (Total)	75		Sum Set (Total)	31		Sum Set (Total)	46		Sum Set (Total)	636
	Sum Set (Up)	59		Sum Set (Up)	22		Sum Set (Up)	23		Sum Set (Up)	636
	Sum Set (Dn)	16		Sum Set (Dn)	9		Sum Set (Dn)	23		Sum Set (Dn)	0
	PCP NOT Sum	23		PCP NOT Sum	24		PCP NOT Sum	81		PCP NOT Sum	556

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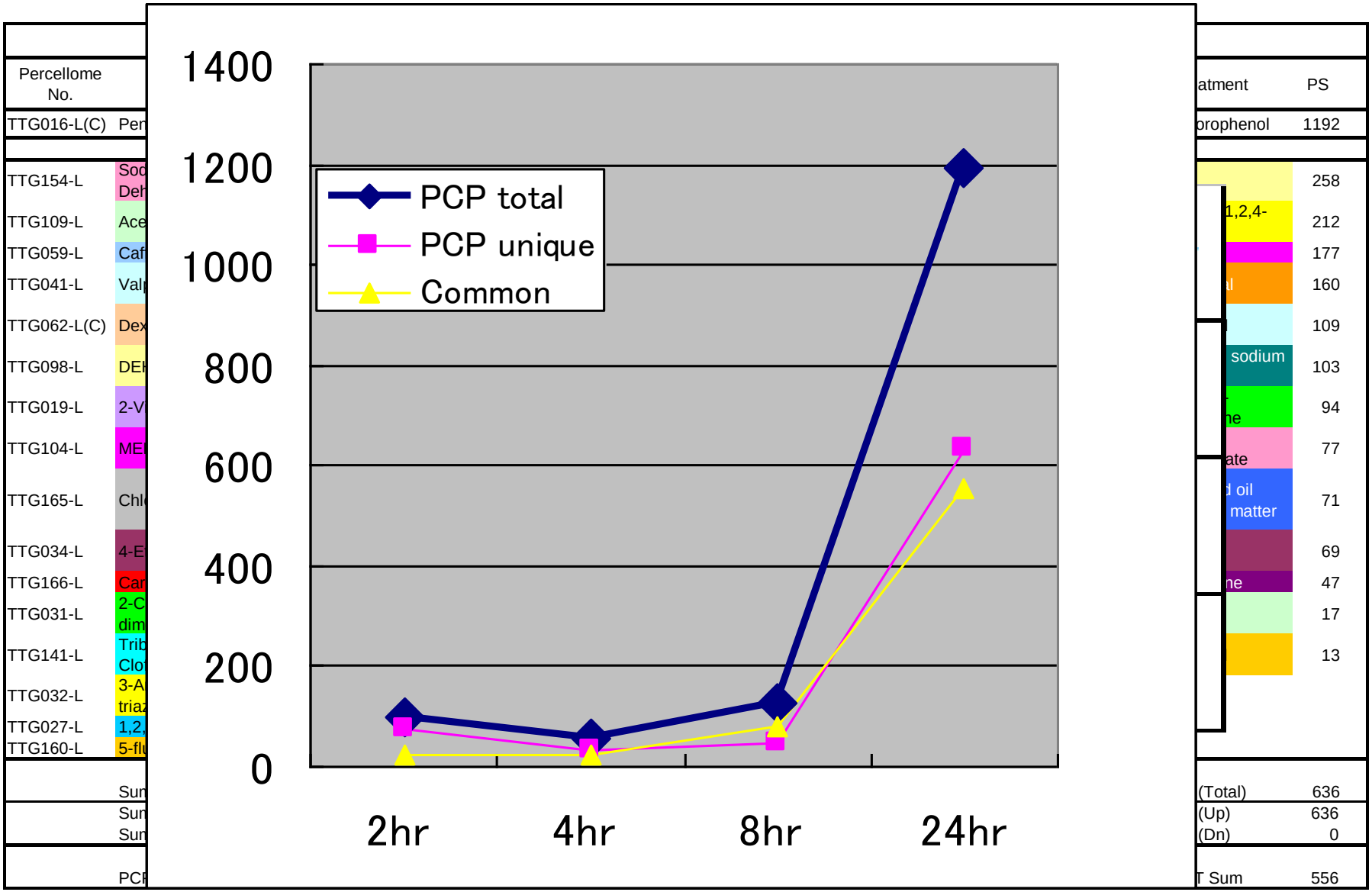

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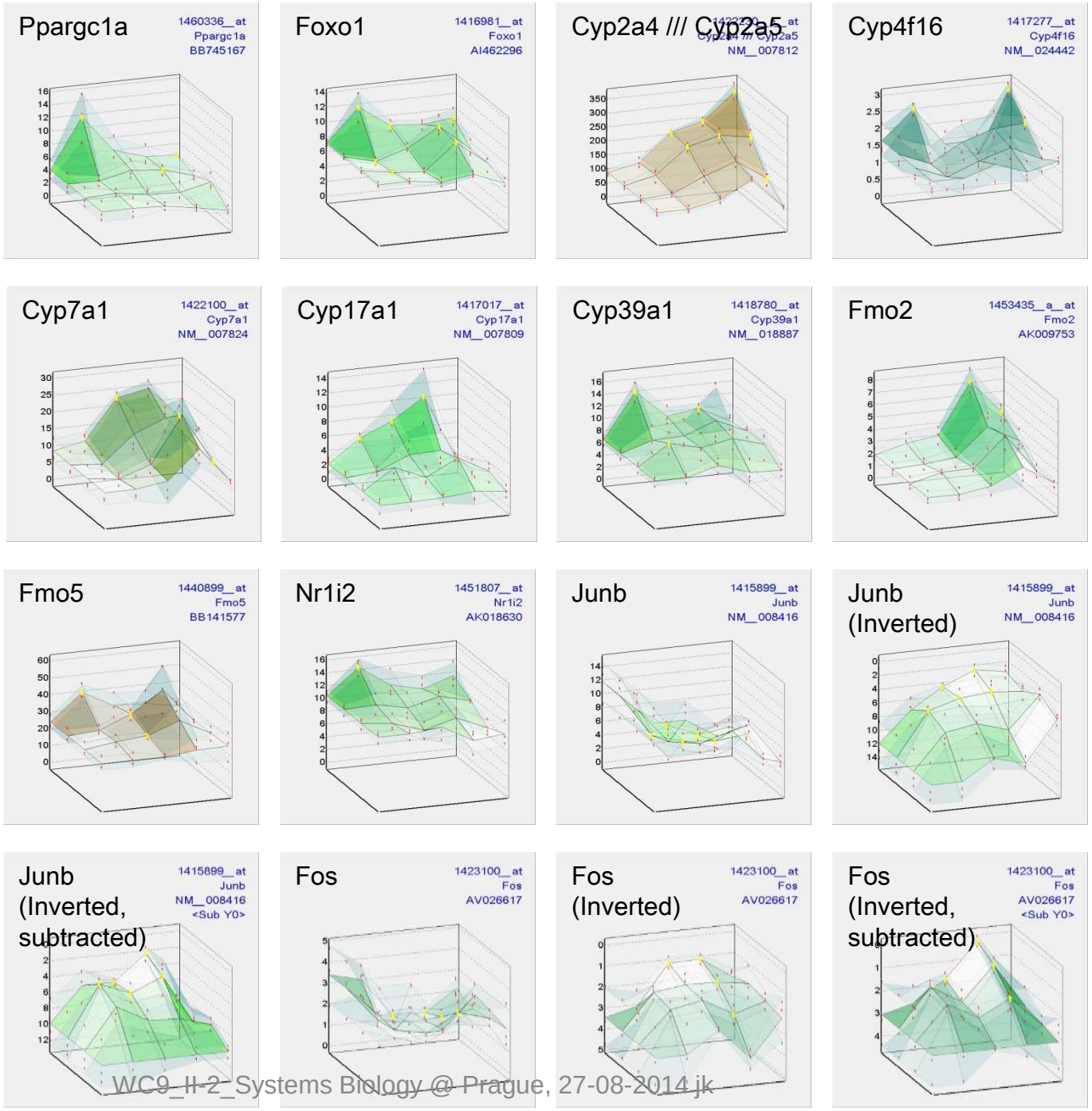
Genes Induced at 2, 4 or 8 hr



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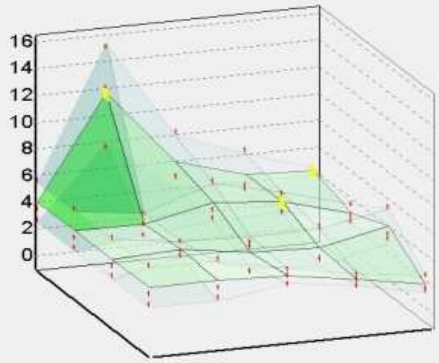
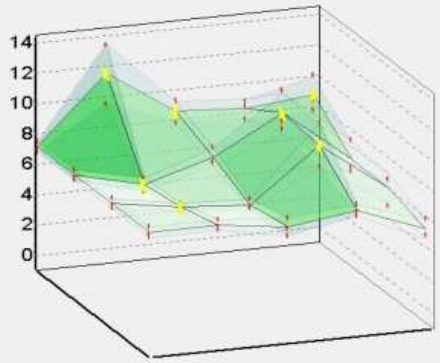
2, 4 or 8 hr

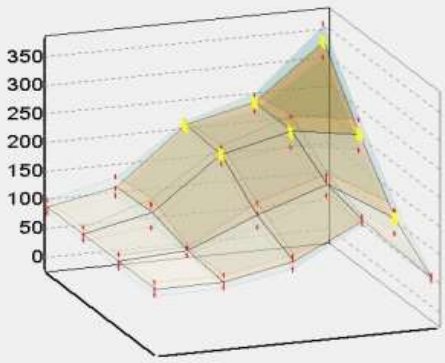
Ppargc1a
1460336_at
Ppargc1a
BB745167



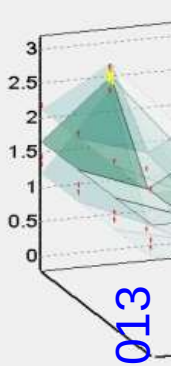
Foxo1
1416981_at
Foxo1
AI462296



Cyp2a4 /// Cyp2a5
1422230_s_at
Cyp2a4 /// Cyp2a5
NM_007812



Cyp4f16



Cyp7a1
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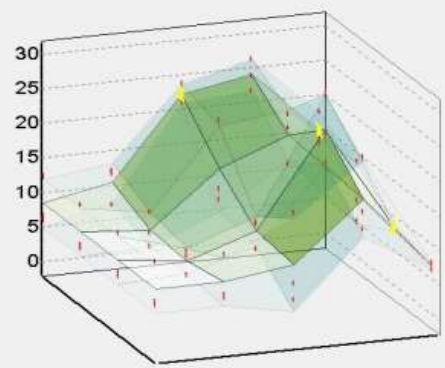

Cyp17a1
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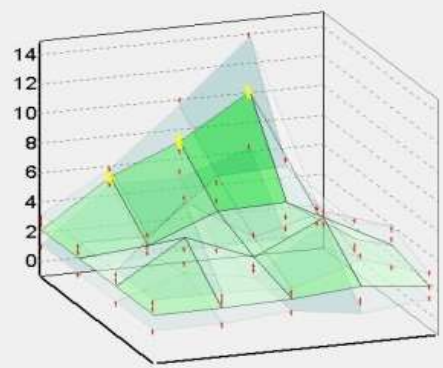

Cyp39a1
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Cyp39a1
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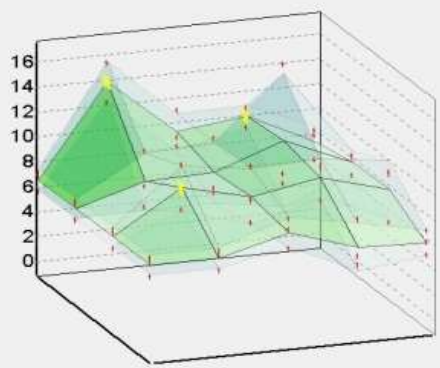
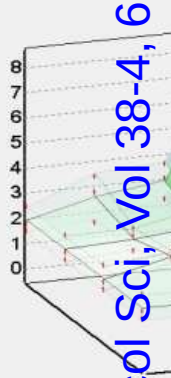


Fmo2



Fmo5
1440899_at
Fmo5
BB141577

Nr1i2
1451807_at
Nr1i2
AK018630

Junb
1415899_at
Junb
NM_008416

Junb
(Inverted)

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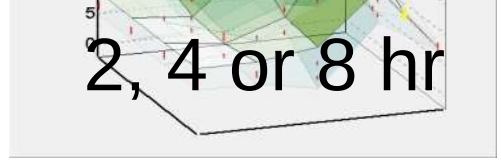
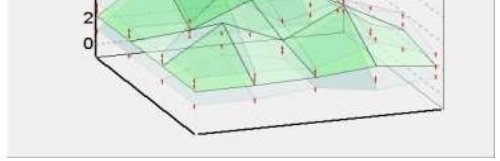
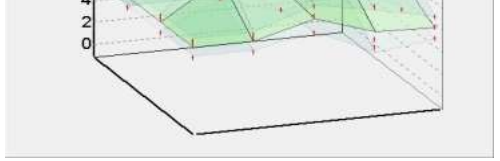
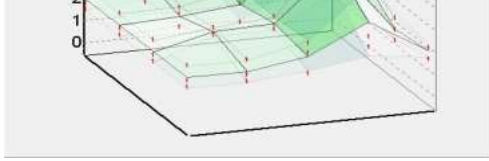
2, 4 or 8 hr









Fmo5

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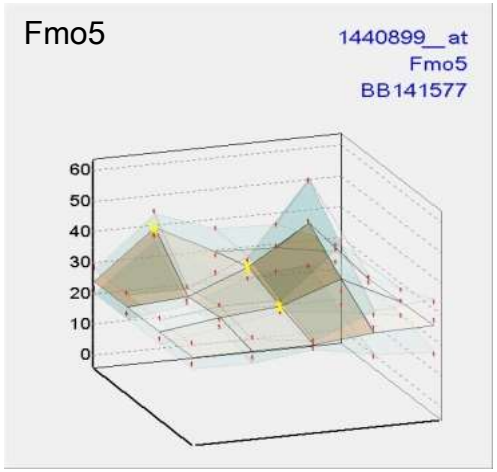
Fmo5
BB141577



Nr1i2

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Nr1i2
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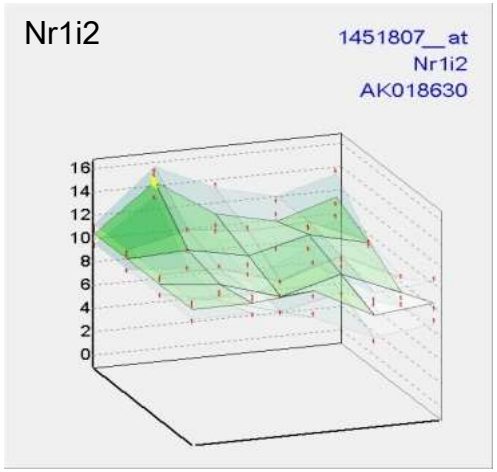
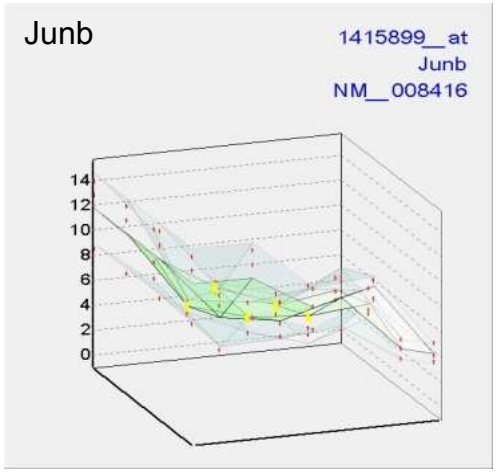


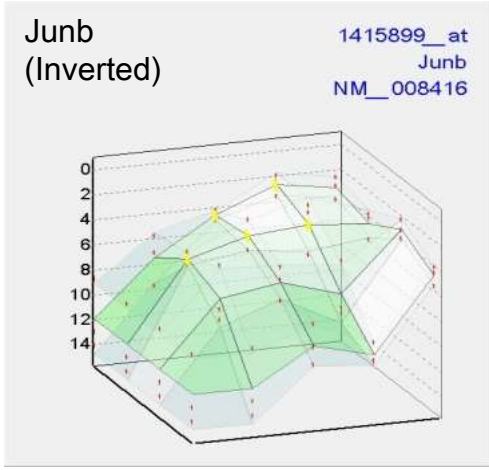
Junb

1415899_at
Junb
NM_008416



Junb
(Inverted)

1415899_at
Junb
NM_008416



Junb
(Inverted, subtracted)

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Junb
NM_008416
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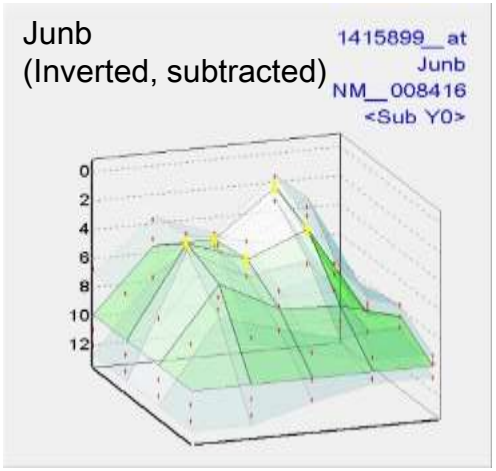
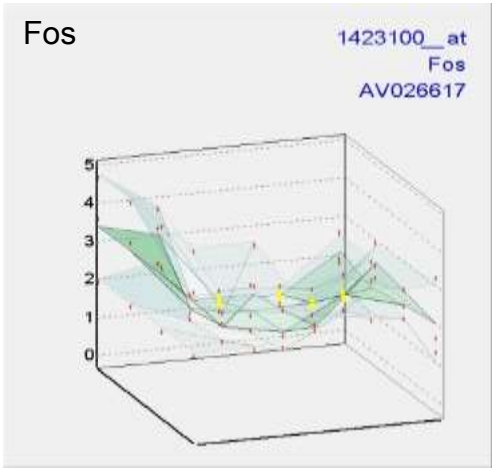


Fos

1423100_at
Fos
AV026617



Fos
(Inverted)

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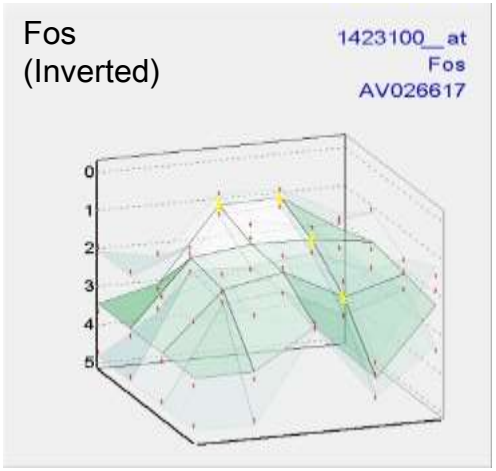

Fos
(Inverted, subtracted)

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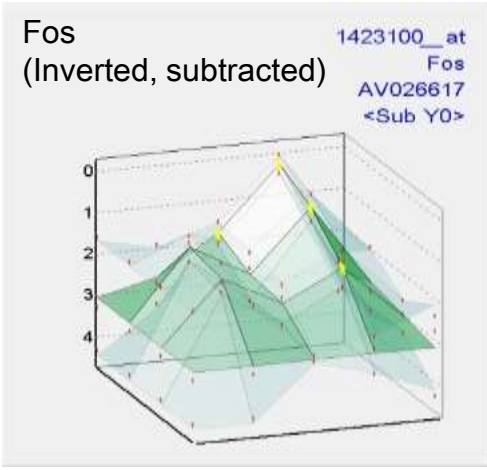

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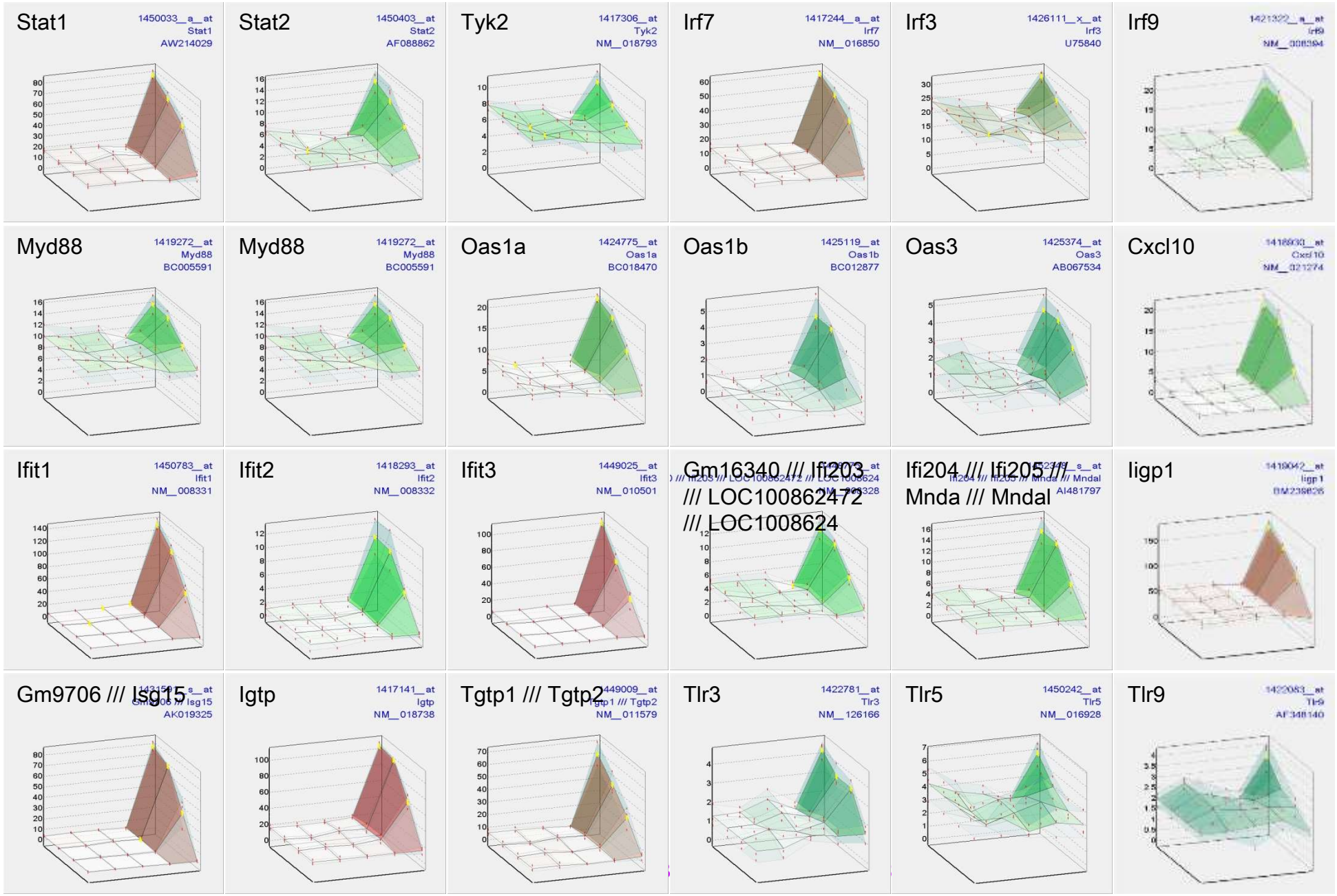
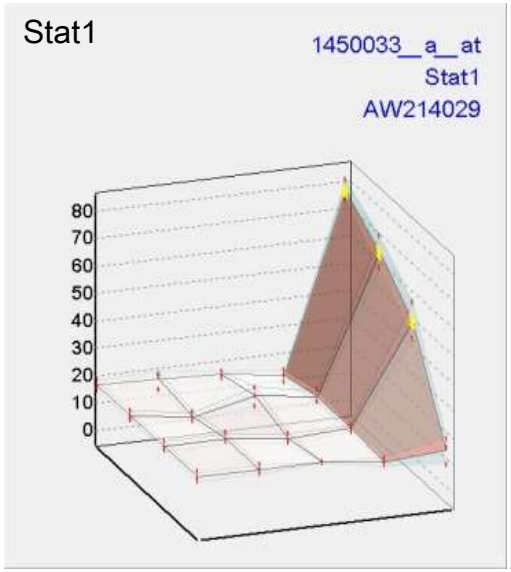
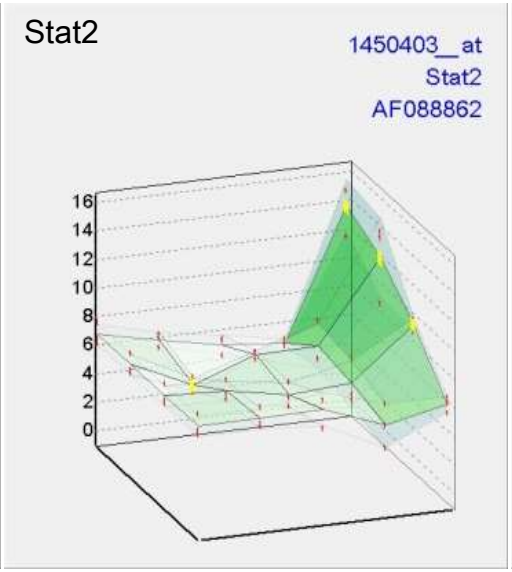
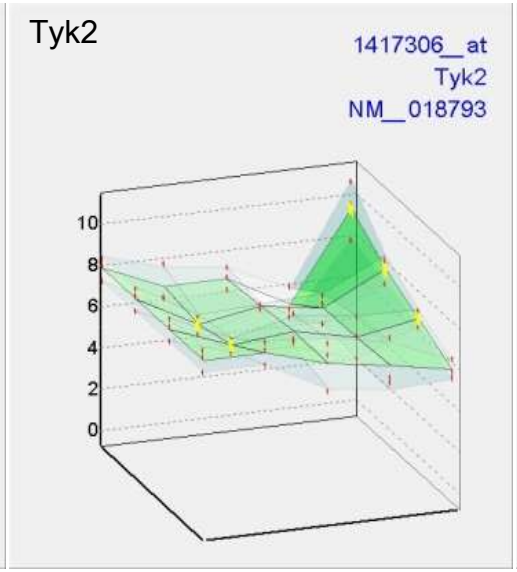
Genes induced at 24 hr (unique to PCP)

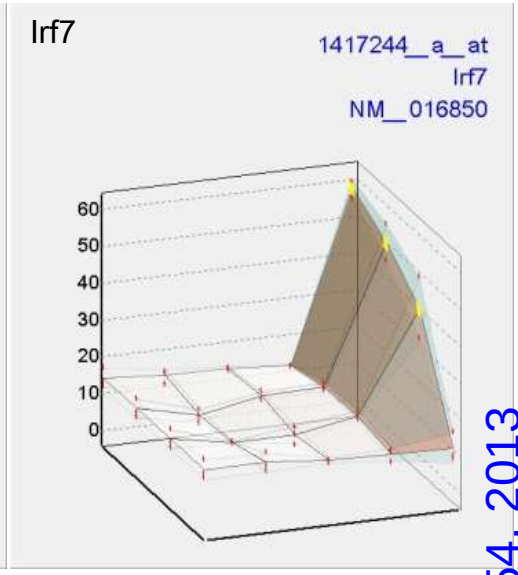


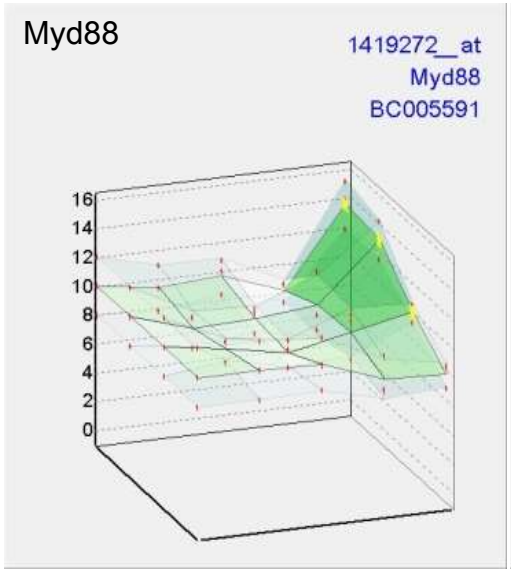
Genes induced at 24 hr (unique to PCP)

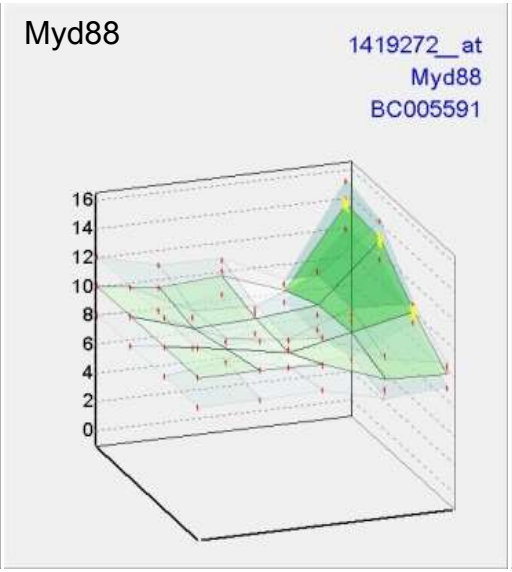


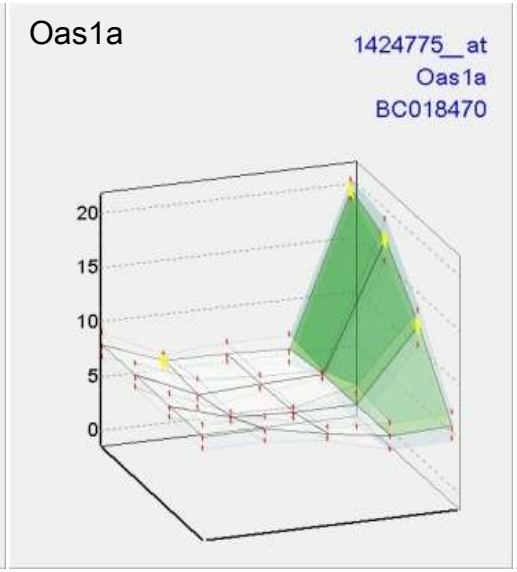


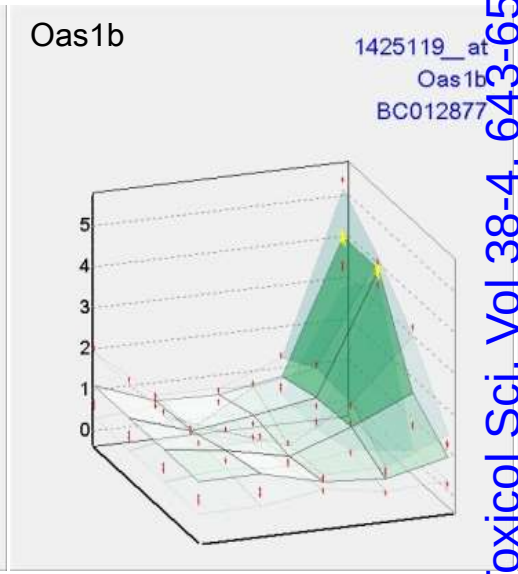


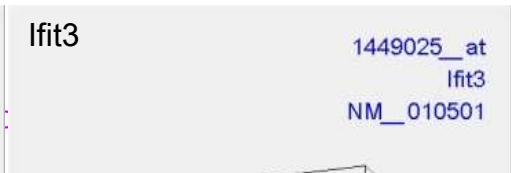


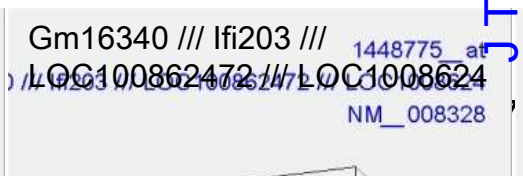












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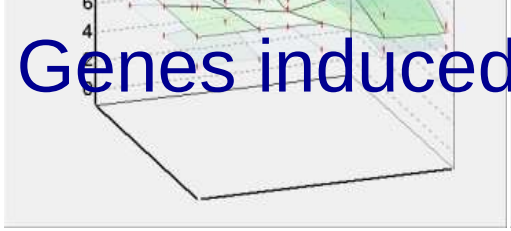
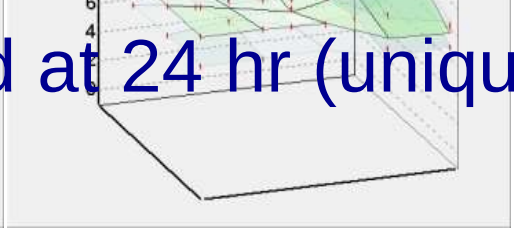
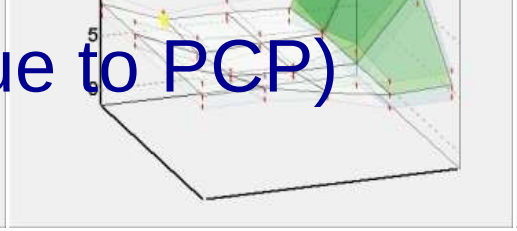
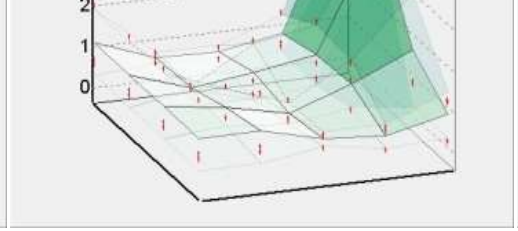
Genes induced at 24 hr (unique to PCP)



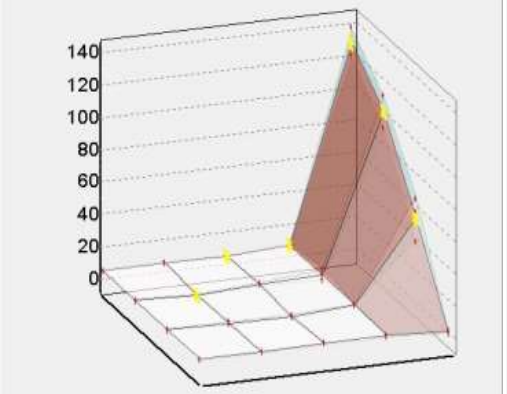






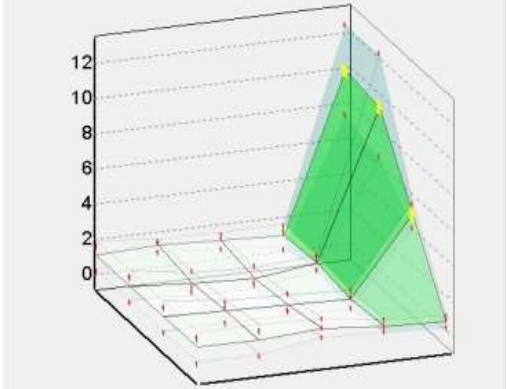
Ifit1

1450783_at
Ifit1
NM_008331



Ifit2

1418293_at
Ifit2
NM_008332



Ifit3

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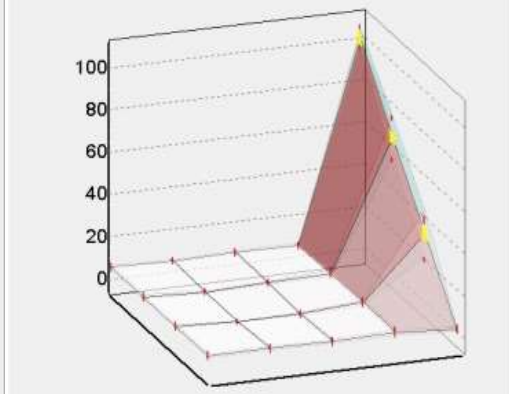
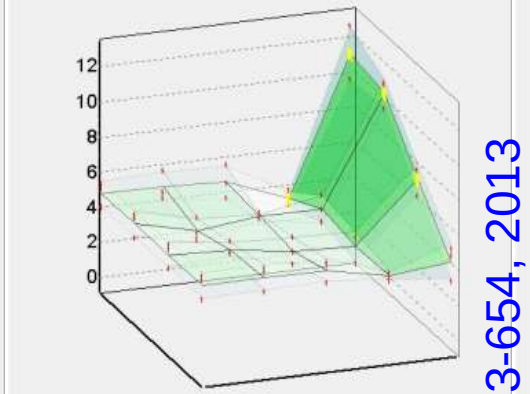
Ifit3
NM_010501



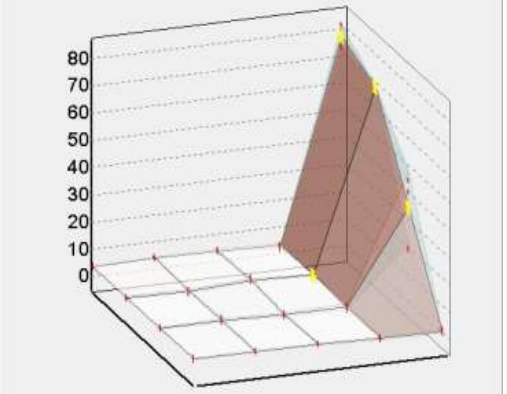
Gm16340 /// Ifi203 /// LOC100862472 /// LOC1008624

1448775_at
NM_008328



Gm9706 /// Isg15

1431591_s_at
Gm9706 /// Isg15
AK019325



Igtp

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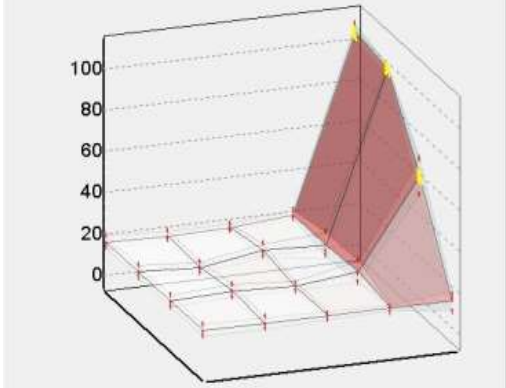
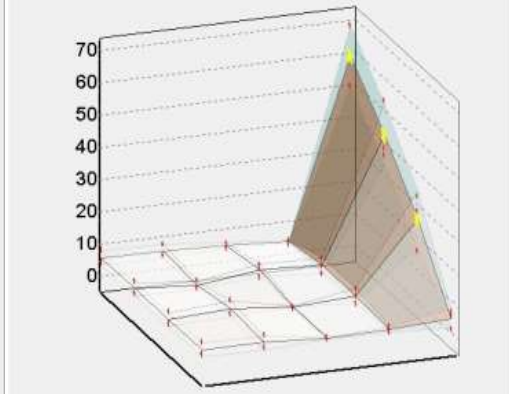
Igtp
NM_018738



Tgtp1 /// Tgtp2

1449009_at
Tgtp1 /// Tgtp2
NM_011579



Tlr3

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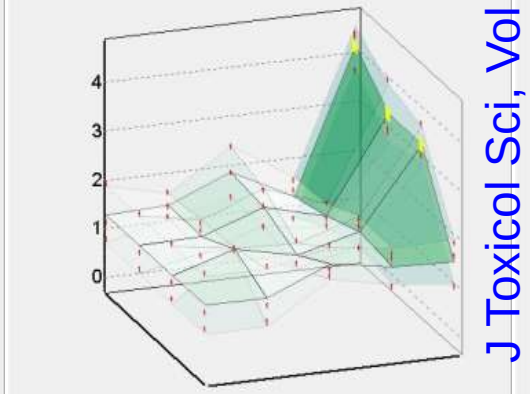
Tlr3
NM_126166



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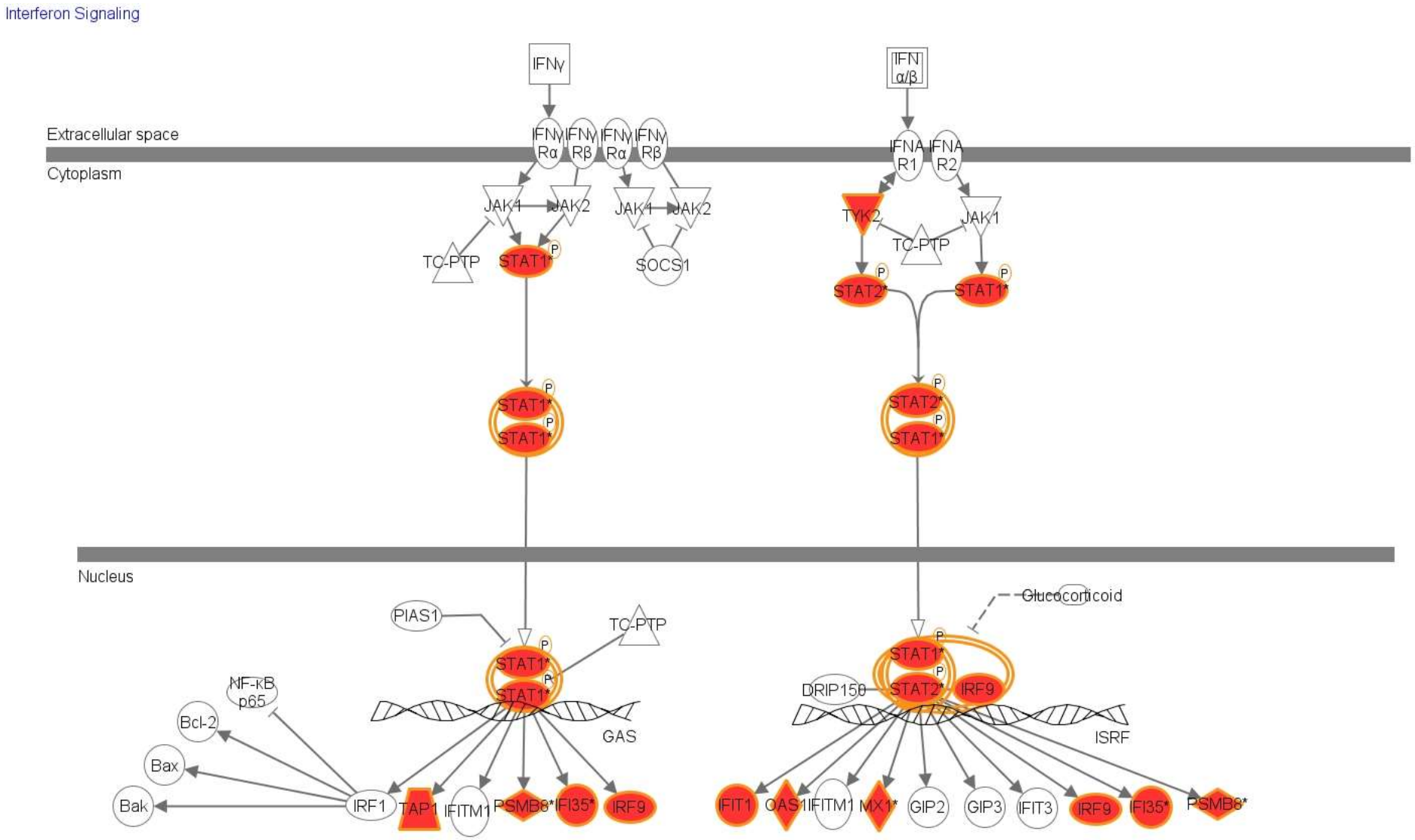
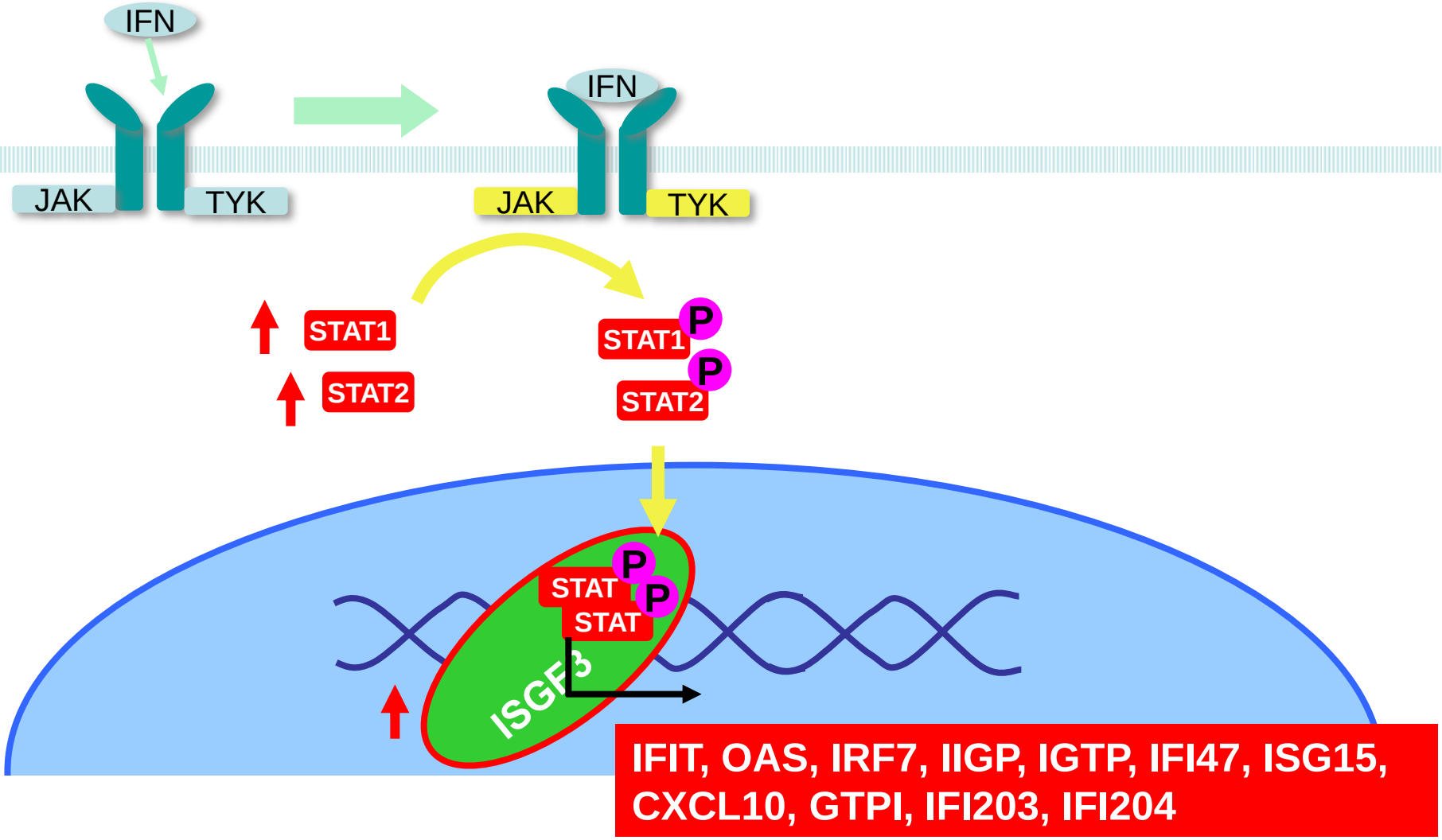
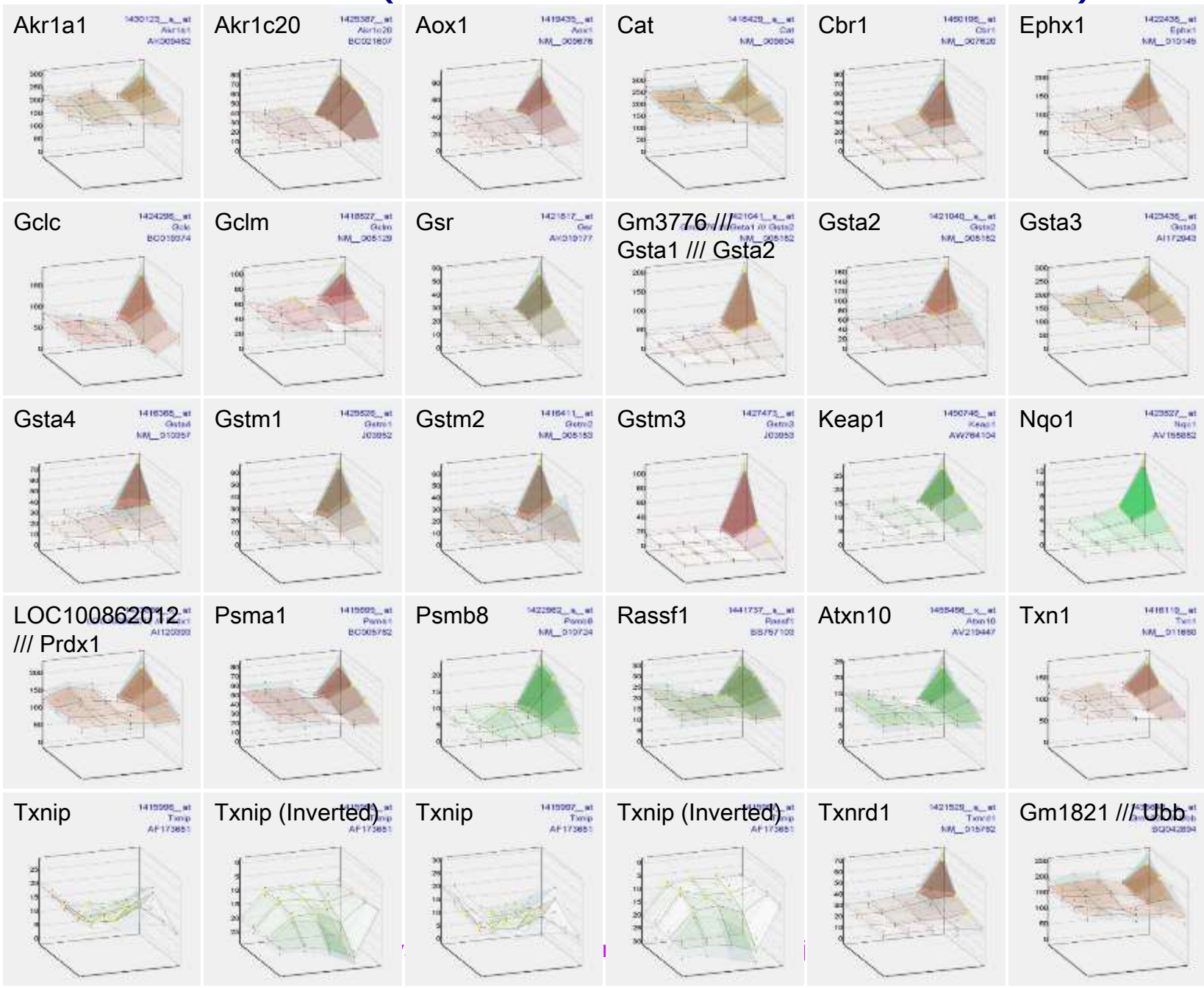
Interferon signaling (from IPA)




IFN
JAK
TYK
IFN
JAK
TYK
STAT1
STAT2
STAT1
P
P
STAT2
P
P
STAT
STAT
ISGF3
IFIT, OAS, IRF7, IIGP, IGTP, IFI47, ISG15,
CXCL10, GTPI, IFI203, IFI204

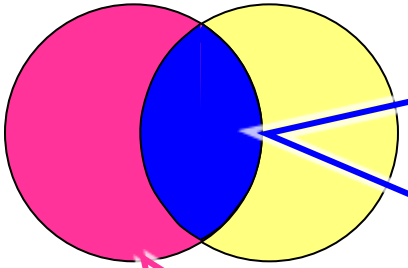
Genes induced at 24 hr (Common to other chemicals)



IPA upstream analysis results for 24hr PCP unique and common to others

PCP

Sum Set of Other chemicals in Percellome DB



NRF2-mediated Oxidative Stress Response	6.3×10^{-18}
Protein Ubiquitination Pathway	3.0×10^{-14}
Xenobiotic Metabolism Signaling	2.2×10^{-05}
Cell Cycle Control of Chromosomal Replication	5.7×10^{-05}
Glutathione-mediated Detoxification	7.1×10^{-05}

Interferon Signaling	4.6×10^{-08}
Activation of IRF by Cytosolic Pattern Recognition Receptors	1.2×10^{-05}
Role of PKR in Interferon Induction and Antiviral Response	2.9×10^{-05}
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	6.4×10^{-05}
IL-1 Signaling	2.2×10^{-04}

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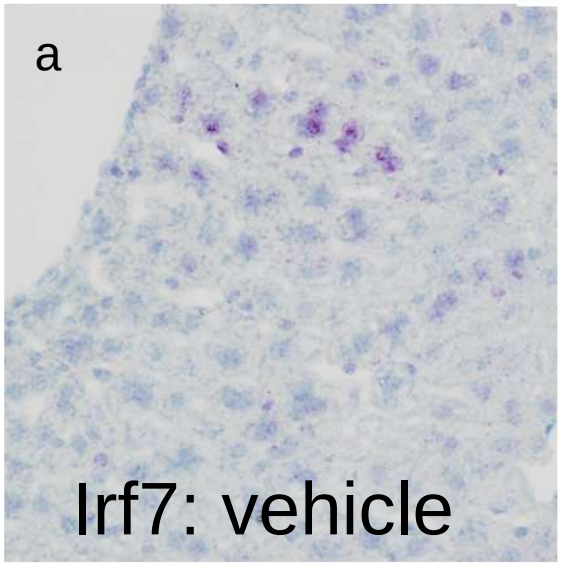
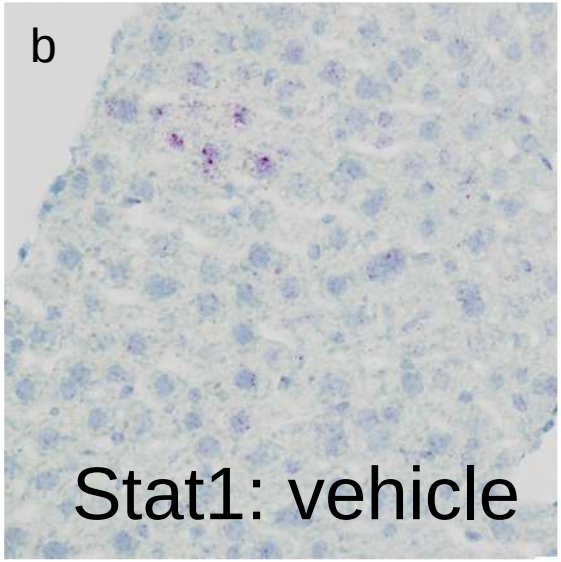
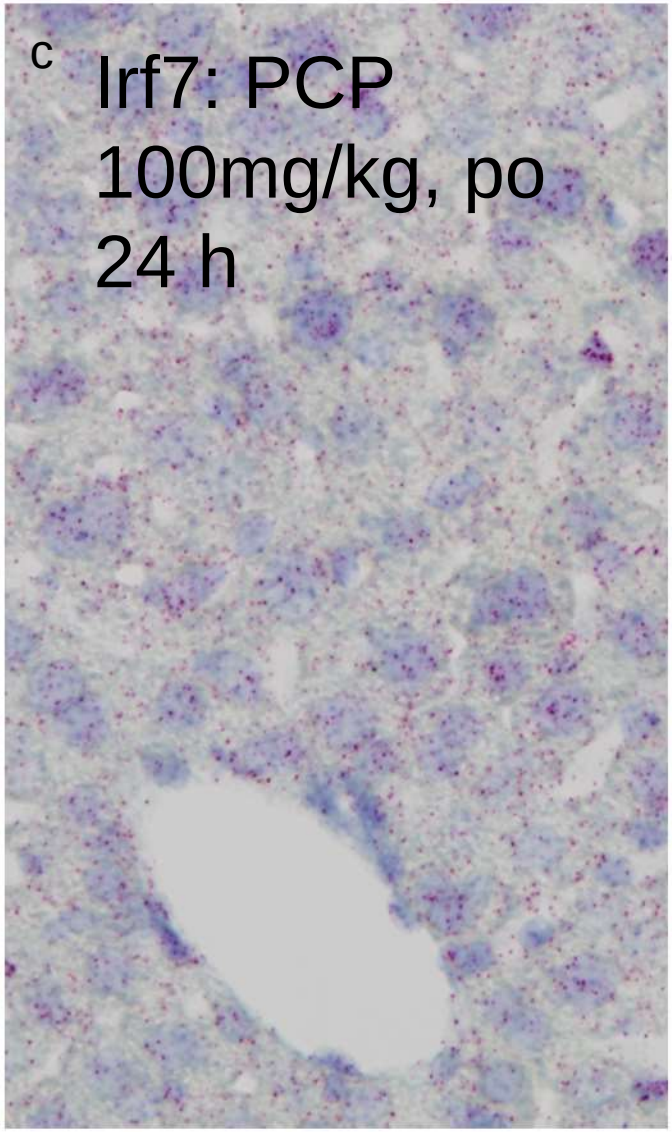
In situ hybridization; QuantiGeneViewRNA ISH Tissue Assay kit (Affymetrix, Inc.)







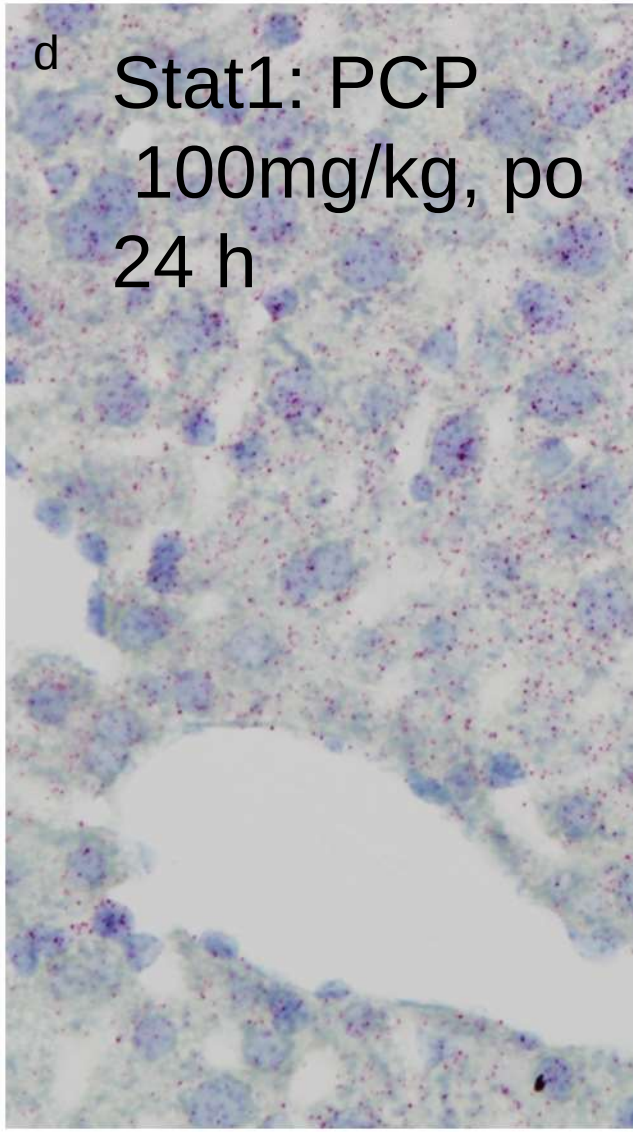


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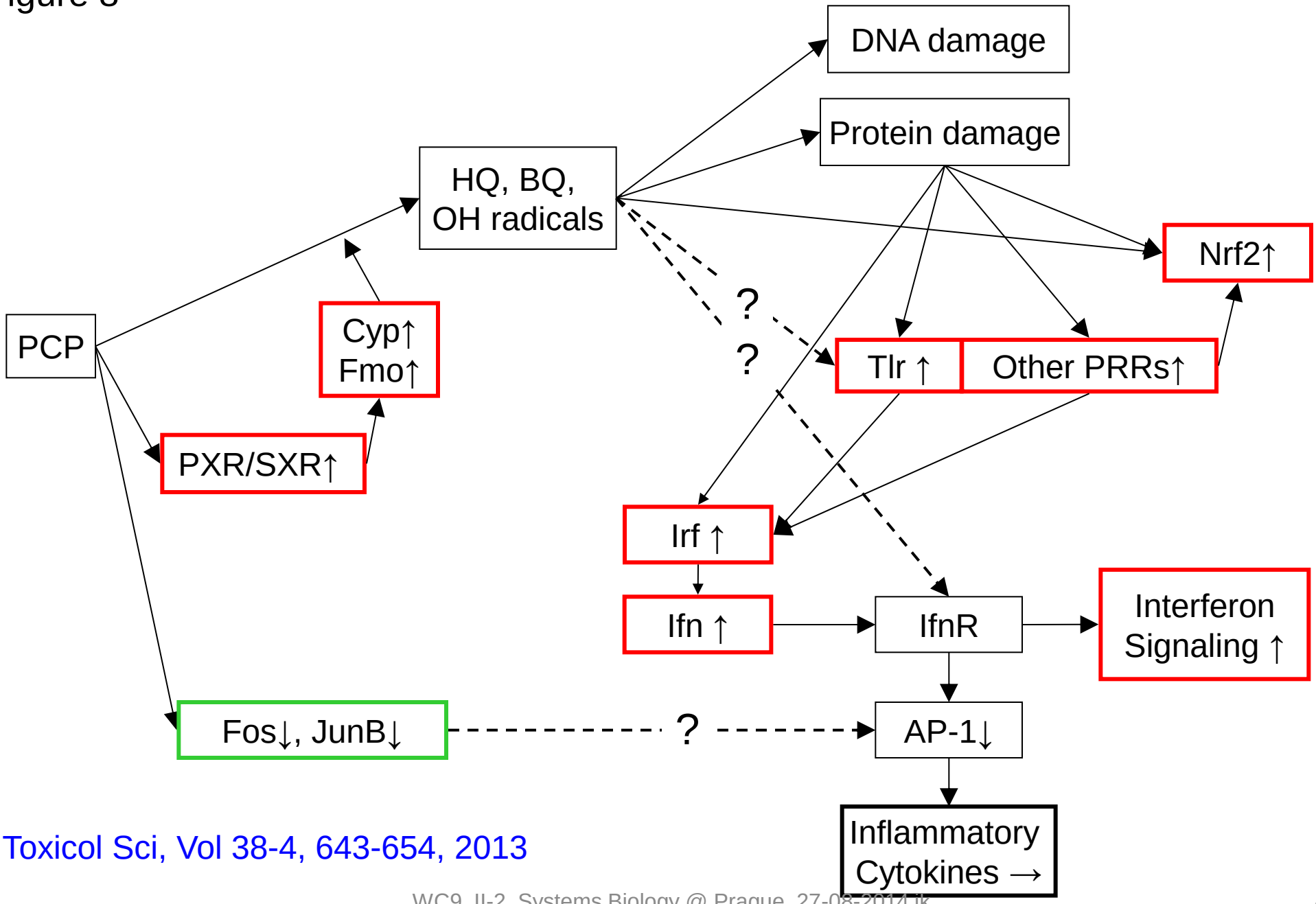
Figure 8



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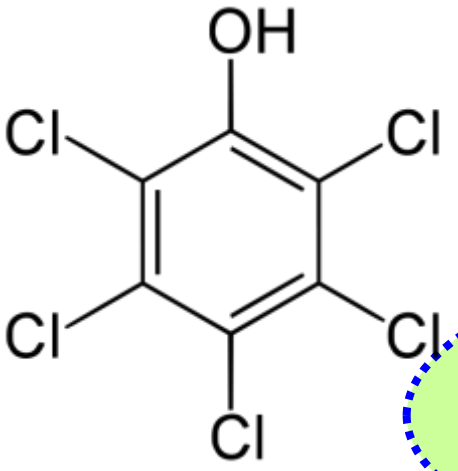
Pentachlorophenol (PCP)



Used as
herbicide,
insecticide,
fungicide,
disinfectant,
and other
preservative
purposes

Classical explanation
= mitochondrial uncoupling

Acute symptoms

Morphological changes as
Damages to liver, kidney, hematology, GI tract

influenza-like symptom

Functional changes as
Hyperthermia, profuse sweating, nausea, uncoordinated movements, etc.

Chronic symptoms:
Liver, kidney, neural
Liver tumor induction

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New Concept of Repeated Dose

Gene Knock-out Mouse

AhRKO
p53 KO
ERαKO
ERβKO
others

Gene Knock-In Mouse

SXR/PXR Humanized mouse

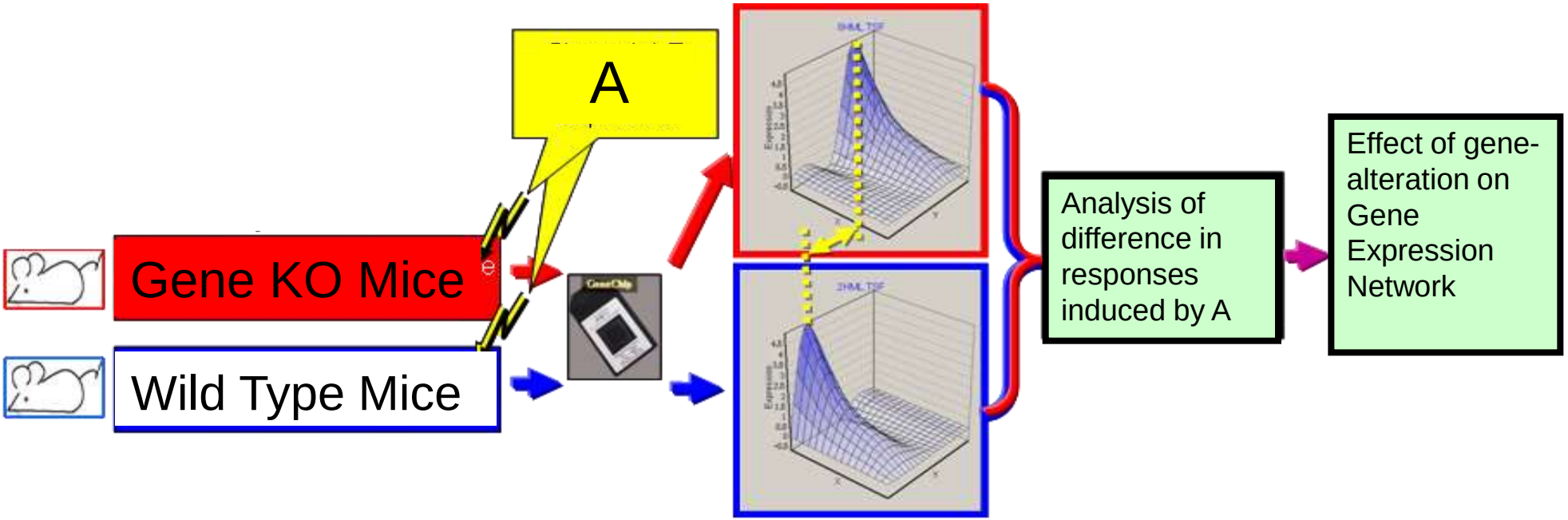
The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.37, No.2, 373-380, 2012

Original Article

Development of humanized steroid and xenobiotic receptor mouse by homologous knock-in of the human steroid and xenobiotic receptor ligand binding domain sequence

Katsuhide Igarashi[1], Satoshi Kitajima[1], Ken-ichi Aisaki[1], Kentaro Tanemura[1], Yuhji Taquahashi[1], Noriko Moriyama[1], Eriko Ikeno[1], Nae Matsuda[1], Yumiko Saga[2,3], Bruce Blumberg[4] and Jun Kanno[1]

Gene Knockout Mice



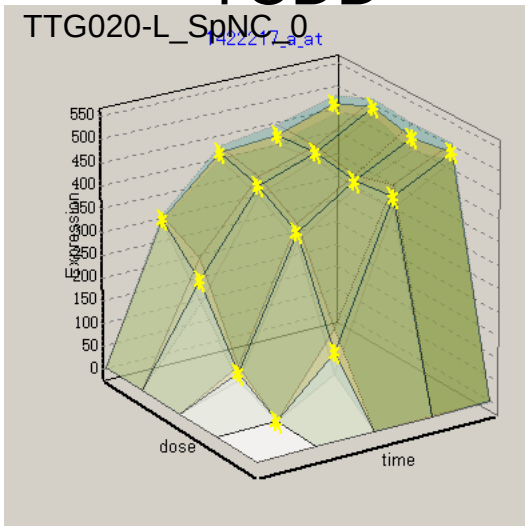
Cyp1a1
NM_009992
cytochrome P450, family 1, subfamily a, polypeptide 1

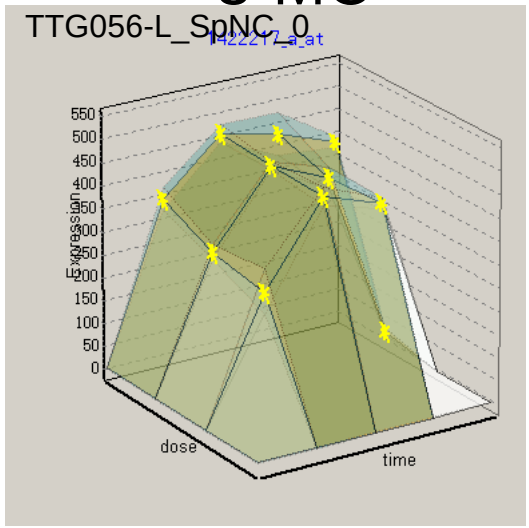
TCDD

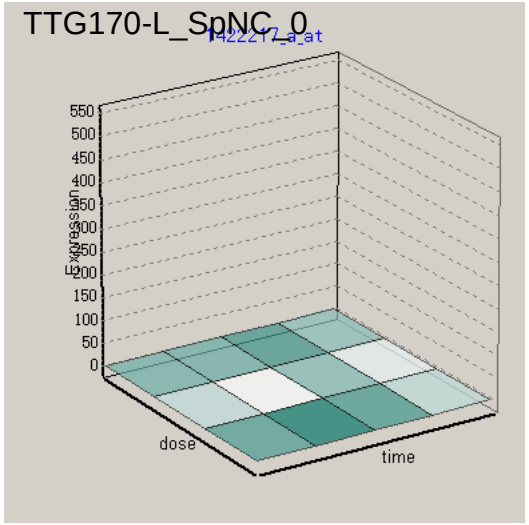
3-MC

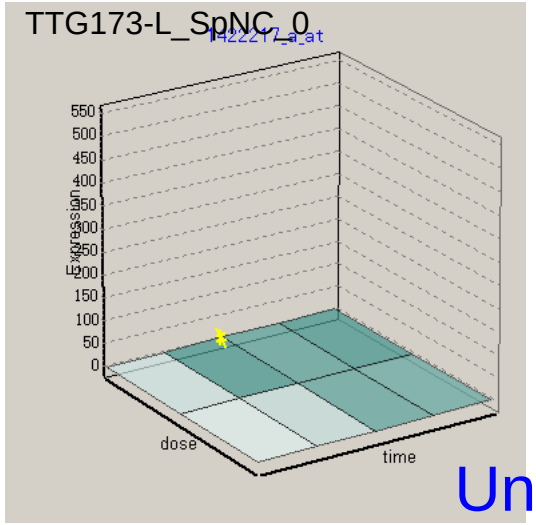
Wild mouse

AhR KO mouse







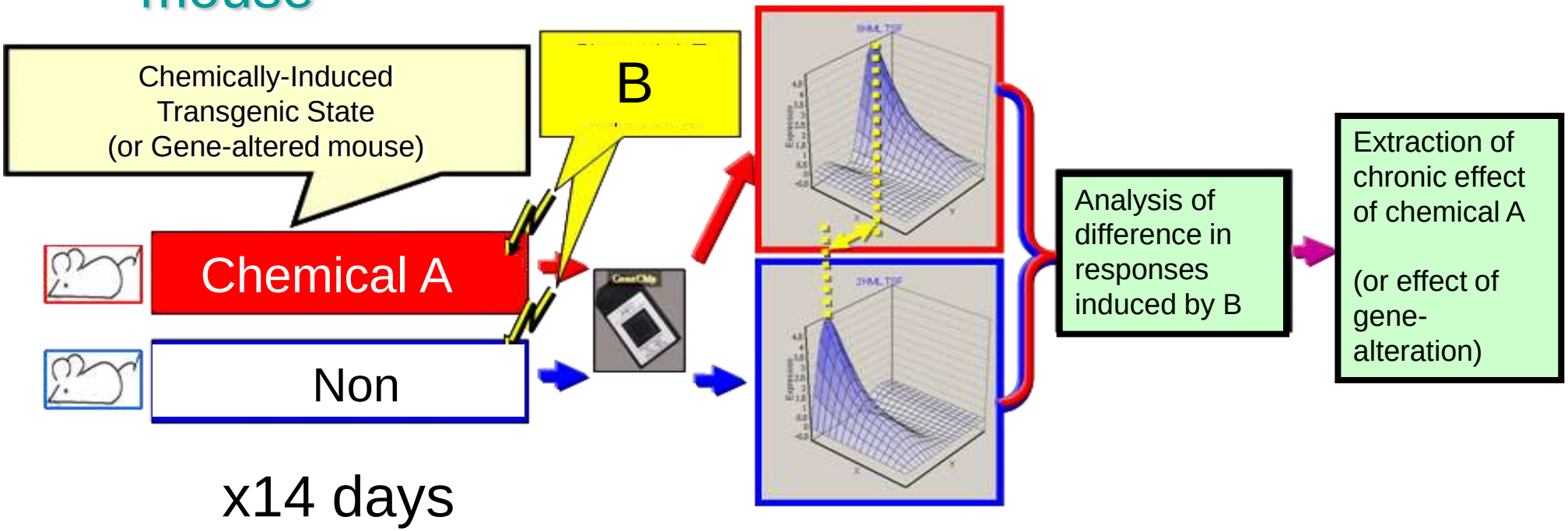


*:T-test p<0.05 compared concurren

Unpublished data

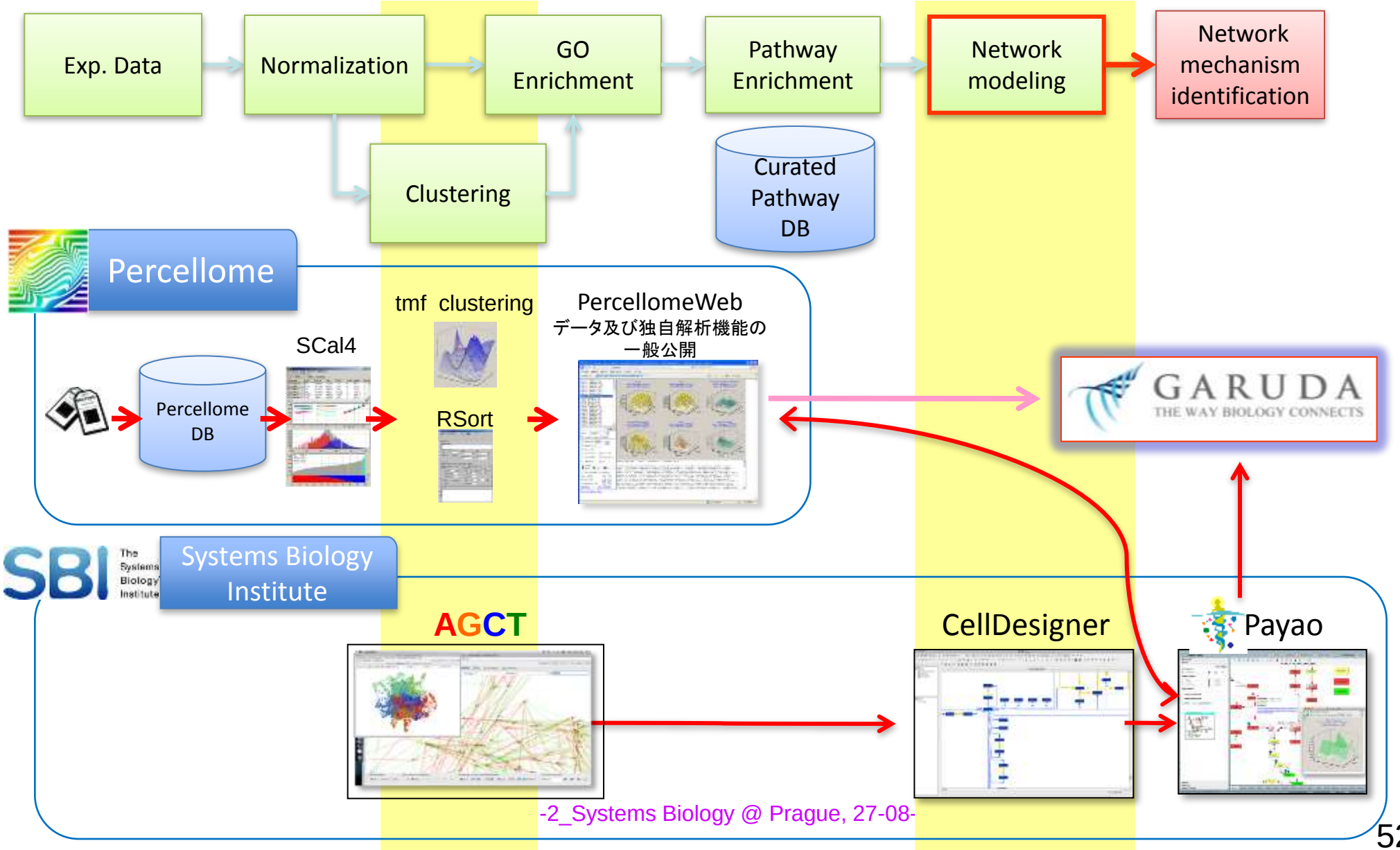
New Concept of Repeated Dose

Consider “chronic/ repeated exposure”
as
“Chemically-Induced Transgenic State”,
which is equivalent to Gene Knockout / transgenic mouse



Whole body inhalation Percellome at Sick-Building-Syndrome Concentration

Gene Network Drawing and International Publication
（with Dr. Hiroaki Kitano, SBI　http://www.sbi.jp/index.htm）



Garuda DashBoard

http://www.garuda-alliance.org/

Percellome Summary

- Ready to Draw dynamic networks with time course
- Merging rat TGP data to mouse Percellome database
- Repeated dose studies reveal fundamental alterations to describe chronic toxicity (data not shown, manuscript in preparation).
- Publish all data to public domain along with all analysis tools via Garuda Project.

Percellome Project: How 3R ?

Conceptually very “3R” !

Ultimate goal: virtual mouse, virtual human
(cf. Star Trek 24th century !)

Less animal, short treatment, at low dose, for the comprensive analysis to predict both acute and chronic toxicity.

Current protocol

n=3 per group
4X4=16 groups, totally 48
Single gavage + 24 hours follow up
Repeated gavage up to 14 days

Near Future protocol

n=3 per group
3x3=9 groups, totally 27
Single gavage + 24 hours follow up
Repeated gavage up to 4 days

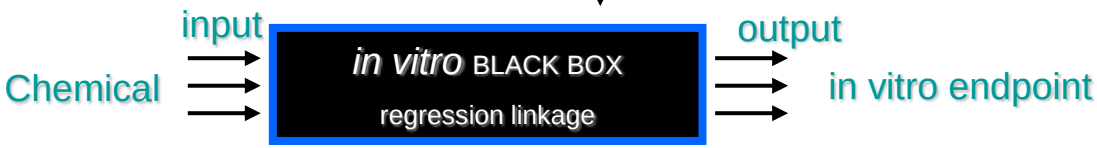
Percellome Project: Contribution to the development of new alternative methods

Two Types of Alternative Methods

In vivo study

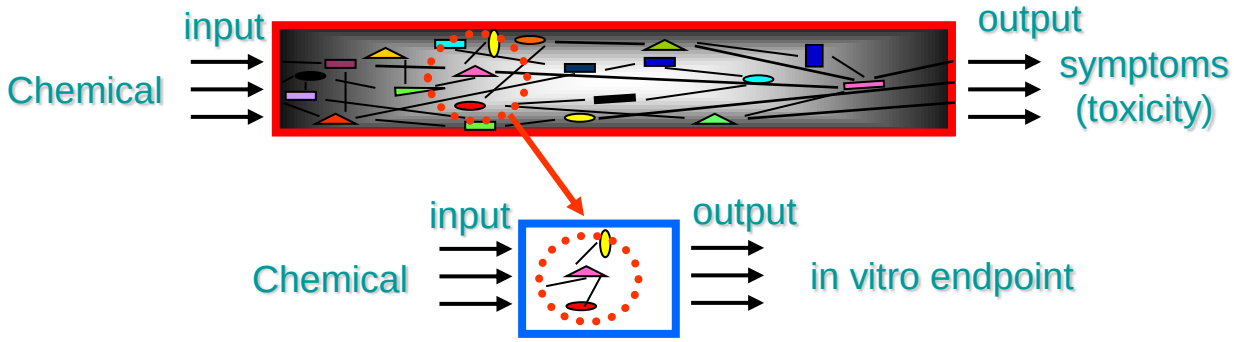
In vivo study is based on Diagnosis of each animal.

(1) Miniature BlackBox Approach



(1) Validation process will be virtually endless.

(2) *In vivo* mechanism Excision Approach



(2) Validation process is easy, using Positive and Negative controls.

Practical Contribution to the development of Alternative Methods

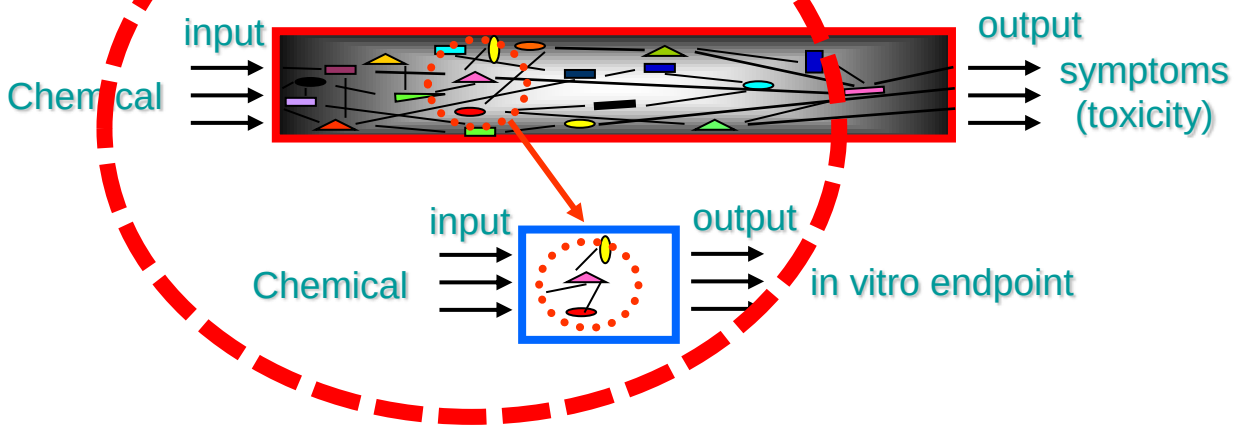
In vivo study

In vivo study is based on Diagnosis of each animal.

(1) Miniature BlackBox Approach

(1) Validation process will be virtually endless.

(2) *In vivo* mechanism Excision Approach



(2) Validation process is easy, using Positive and Negative controls.

Long lasting “Animism // Elemental worship // Nature worship” in Japan (in Asia)

Influencing and Influenced by Buddhism, Confucianism, Shintoism, and others

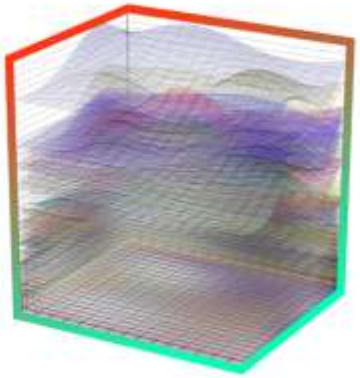
All Japanese Institutions hold Official Annual Ceremony for Thanking the Experimental Animals

Development of Percellome Method (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

NIHS/NIBIO TG Project startup group (~summer 2002) (with 17 Pharm)

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



Systems Biology

Dr. Hiroaki Kitano, SBI
Dr. Natalia Polouliakh, SBI
Dr. Samik Ghosh, SBI

Grants

Ministry of Health, Labor, and Welfare (MHLW) Grant-in-Aid, & MOE

Millefeuille Softwares

Ken-ichi Aisaki, MD, PhD

IT collaboration

NTT COMWARE,
NTT Data
with Teradata, NCR
(Shinya Matsumoto, PhD, Ikuo-ichi Tajima, MD)

Percellome Projects（2003~）In Tox Div NIHS

Jun Kanno, MD, PhD
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
Koichi Morita, Mr
Ayako Imai, Ms
Shinobu Watanabe, Ms
Masaki Tsuji, Mr
Yusuke Furukawa, Mr
Maki Otsuka, Ms
Hisako Aihara, Ms
Minobu Hojo, Ms
Rie Katagiri, Ms
Kiyoshi Sekita, DVM
Yukio Ogawa, DVM (Inhalation)
Satoshi Kitajima DVM, PhD (Fetus)
Kentaro Tanemura DVM, PhD
Atsuya Takagi, DVM, PhD
Yuhji Taquahashi, DVM, PhD