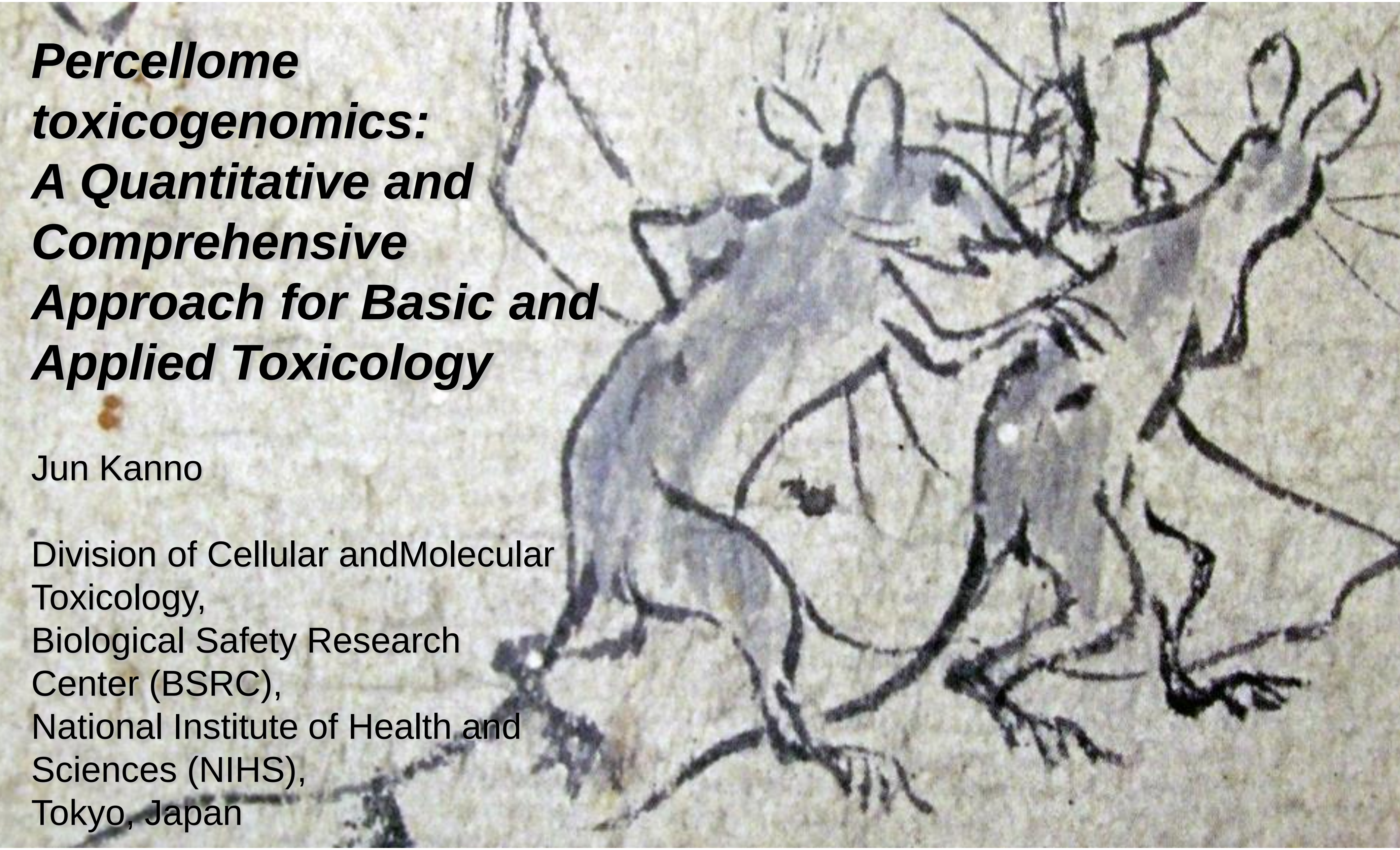


Percellome toxicogenomics: A Quantitative and Comprehensive Approach for Basic and Applied Toxicology

Jun Kanno

Division of Cellular andMolecular Toxicology,
Biological Safety Research Center (BSRC),
National Institute of Health and Sciences (NIHS),
Tokyo, Japan

鳥獣人物戯画より

Contents

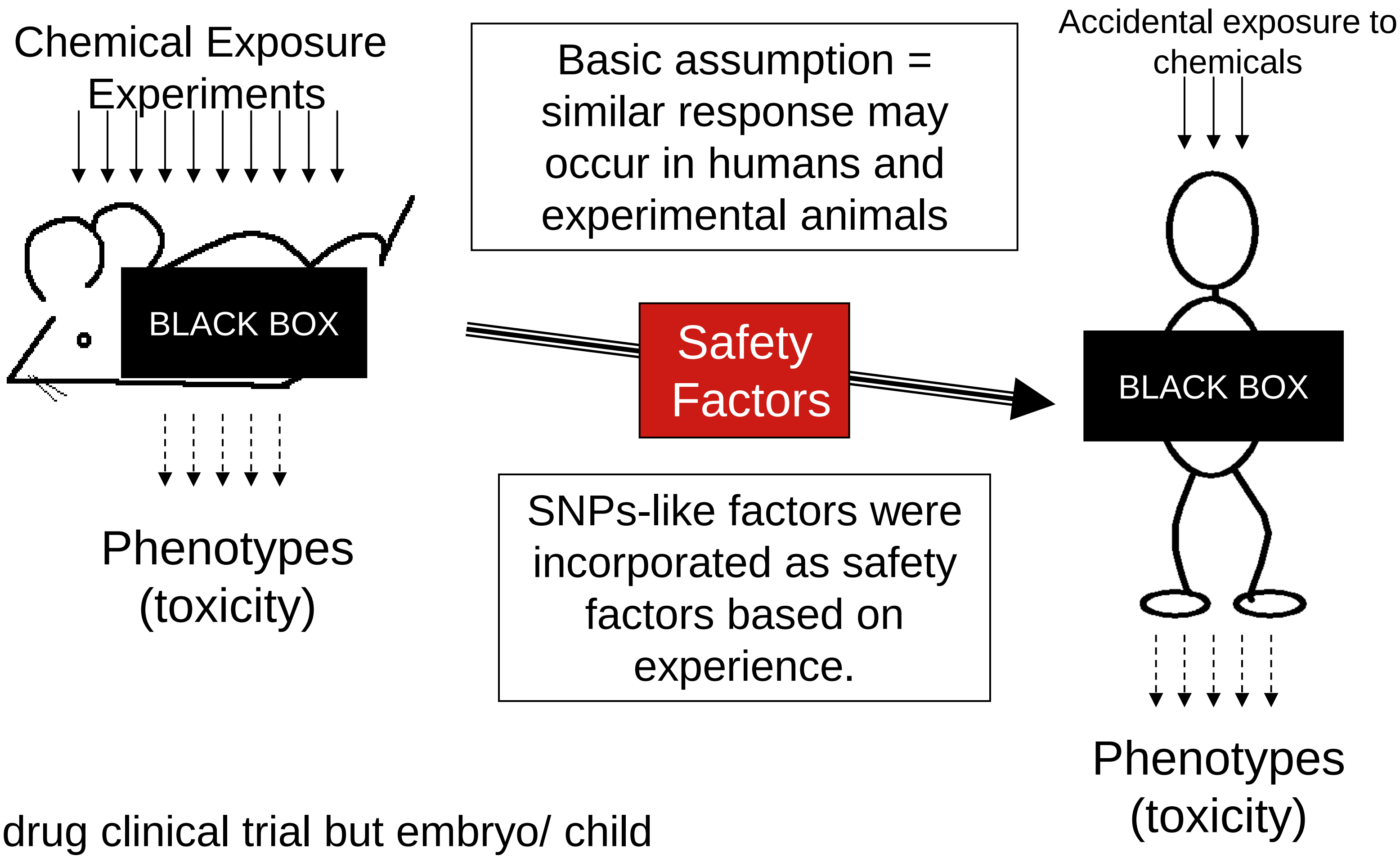
- Percellome Project
  - Backgrounds
  - Analysis examples
    - Estragole (oral gavage) briefly [cf OP2-4]
    - Pentachlorophenol (oral gavage)
  - New concept of repeated dose
    - Gene KO Mouse example (AhRKO)
    - Valproic acid
    - Carbon tetrachloride
  - Percellome (Mouse) and TGP (Rat) data publication

- Appendix
  - Neon swan
    - Fukushima incidence
    - In the Pond of Toxicology

One immediate question/ paradigm was,

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

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



Chemical Exposure Experiments

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

Accidental exposure to chemicals

Safety Factors

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

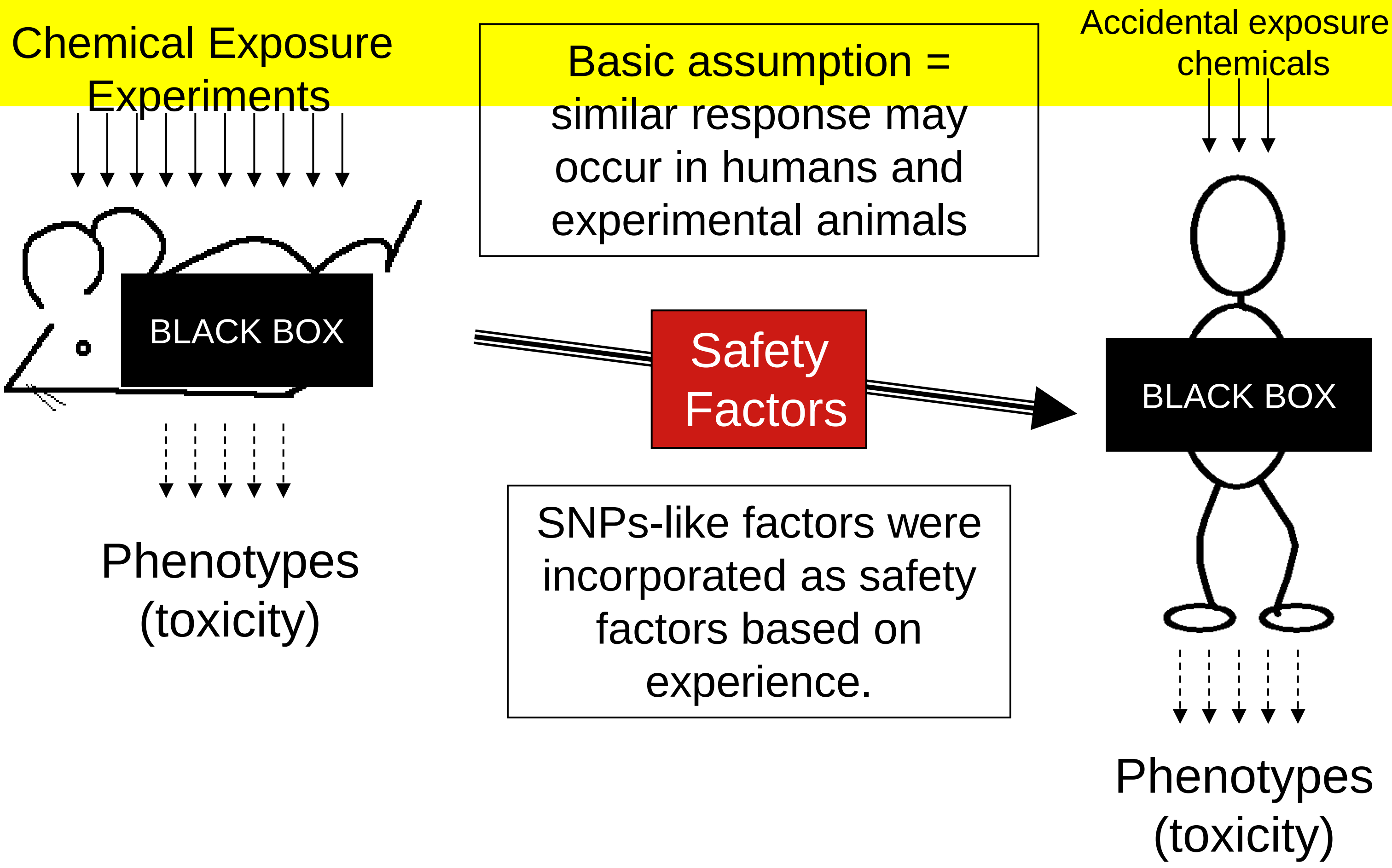
Phenotypes (toxicity)

Phenotypes (toxicity)

Except drug clinical trial but embryo/ child

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

To avoid 2nd "Thalidomide Story"



Chemical Exposure Experiments

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

Accidental exposure to chemicals

Safety Factors

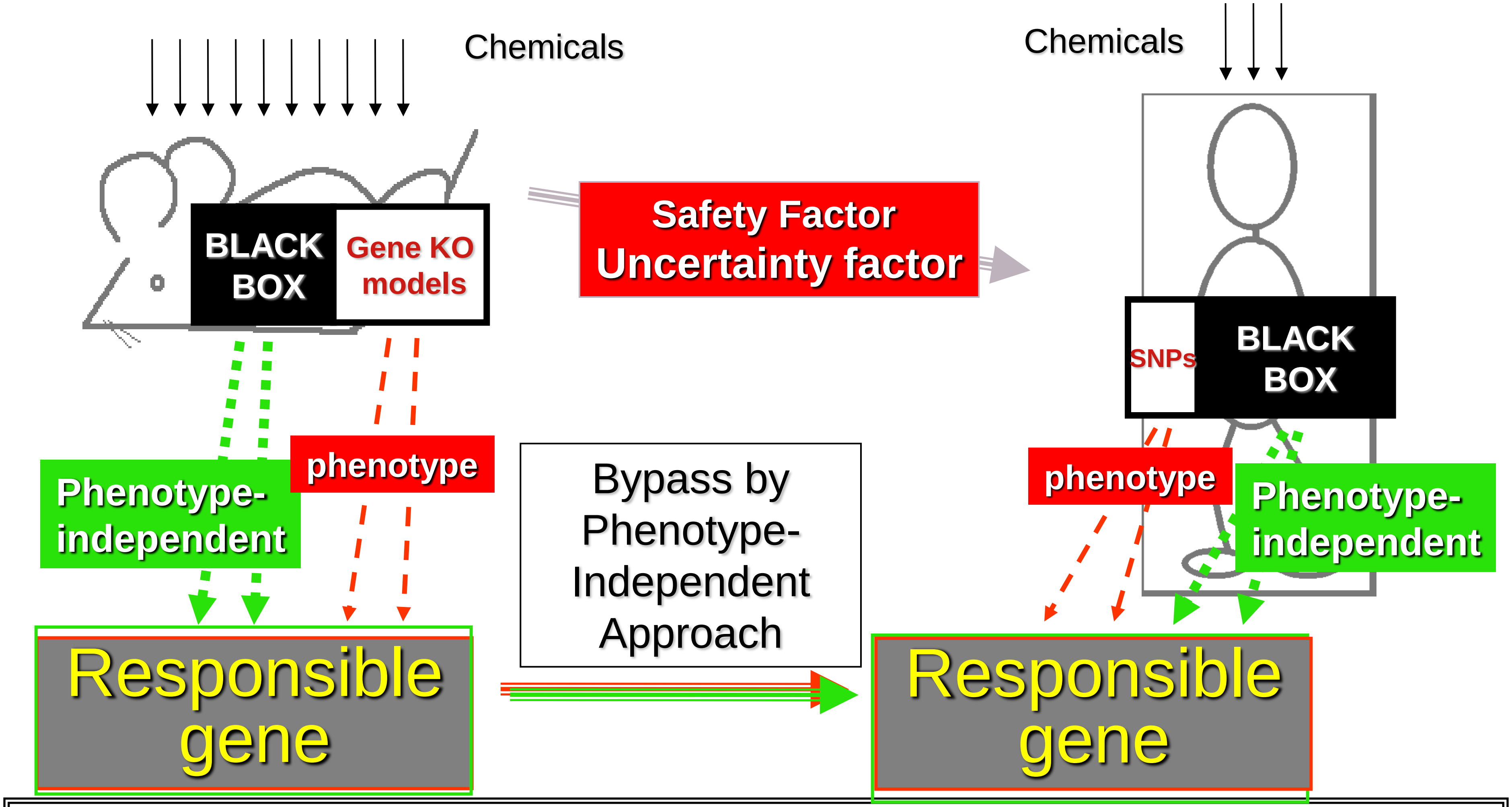
SNPs-like factors were incorporated as safety factors based on experience.

Phenotypes (toxicity)

Phenotypes (toxicity)

Mechanism-based Toxicology

(Phenotype-Independent Approach)



Chemicals

BLACK BOX

Gene KO models

Safety Factor
Uncertainty factor

Chemicals

SNPs

BLACK BOX

phenotype

Phenotype-independent

Bypass by Phenotype-Independent Approach

phenotype

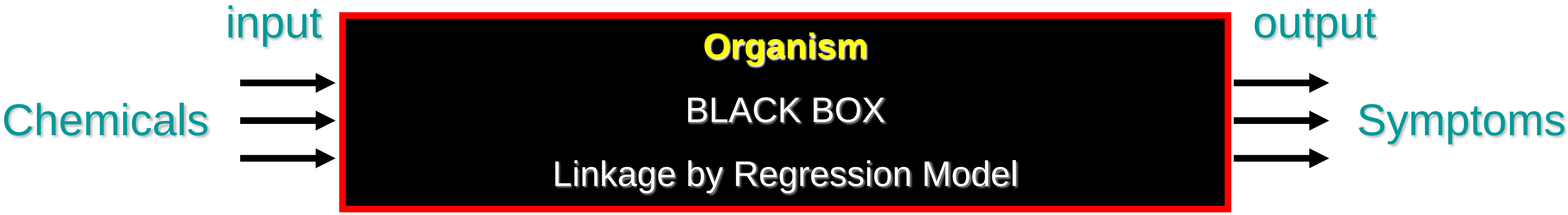
Phenotype-independent

Responsible gene

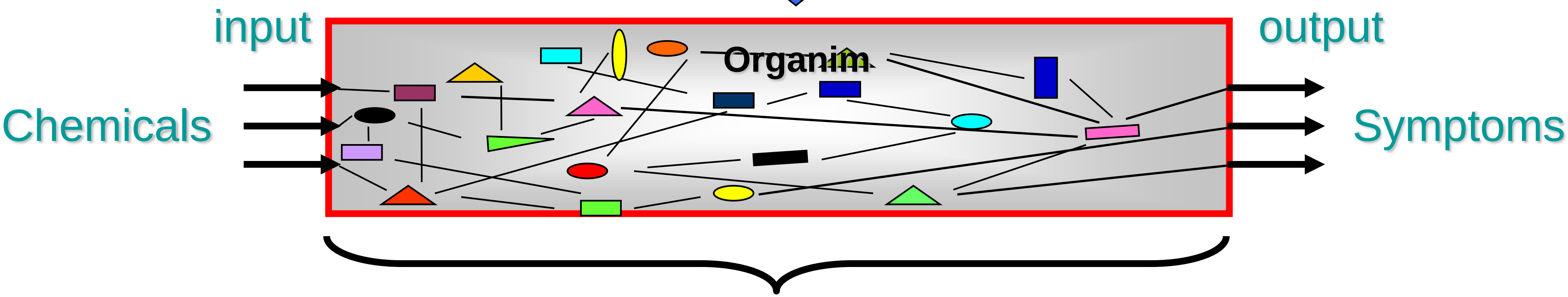
Responsible gene

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

Why monitor all genes comprehensively


input
output
Chemicals
Organism
BLACK BOX
Linkage by Regression Model
Symptoms

Mechanism-based modernization


input
output
Chemicals
Organim
Symptoms

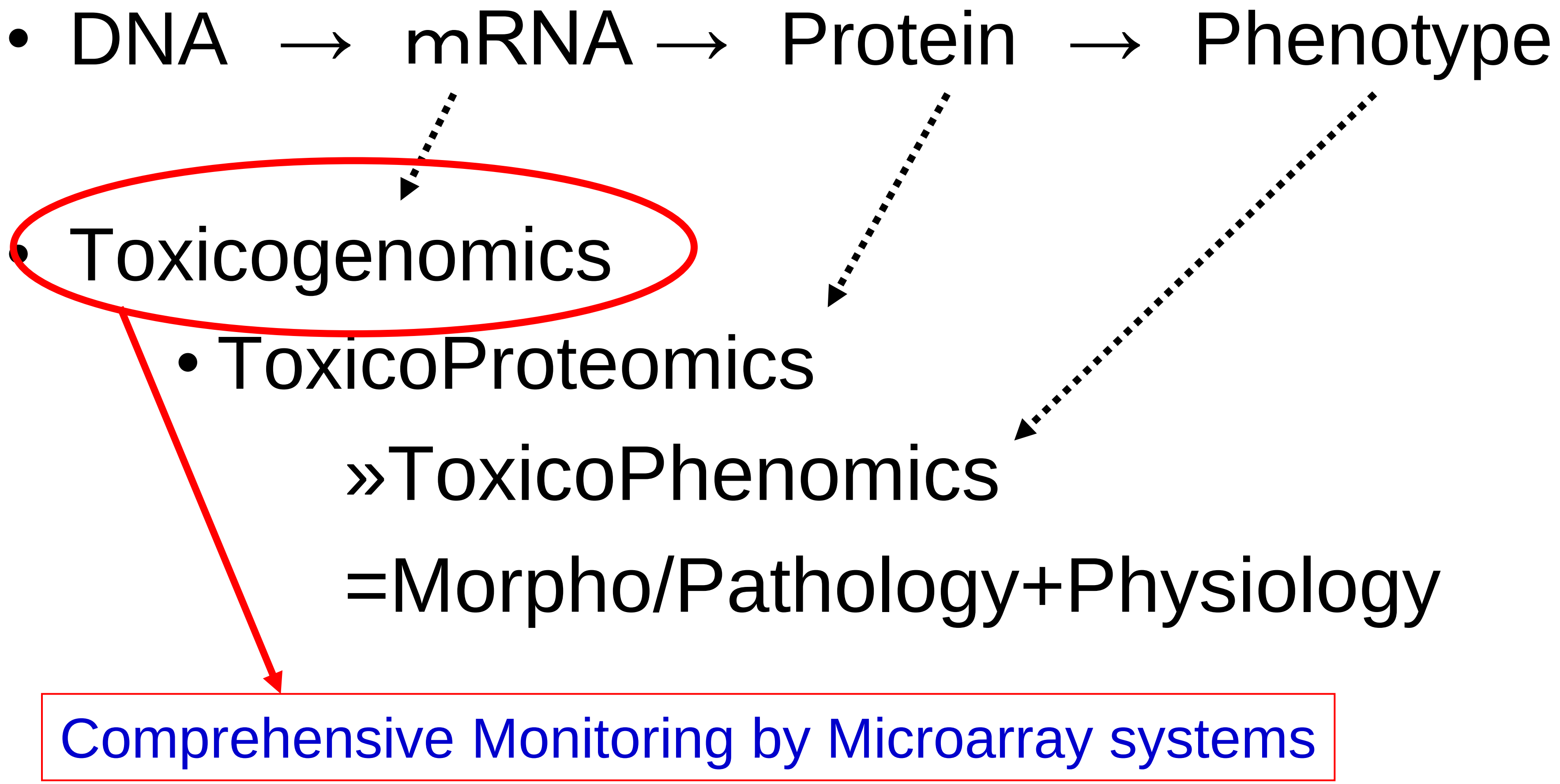
Development of Network Database by _All genes_

(not only “VIP” genes)

↓

Not all networks accompany phenotype

Why mRNA



- DNA → mRNA → Protein → Phenotype

- Toxicogenomics
  - ToxicoProteomics
    - »ToxicoPhenomics

=Morpho/Pathology+Physiology

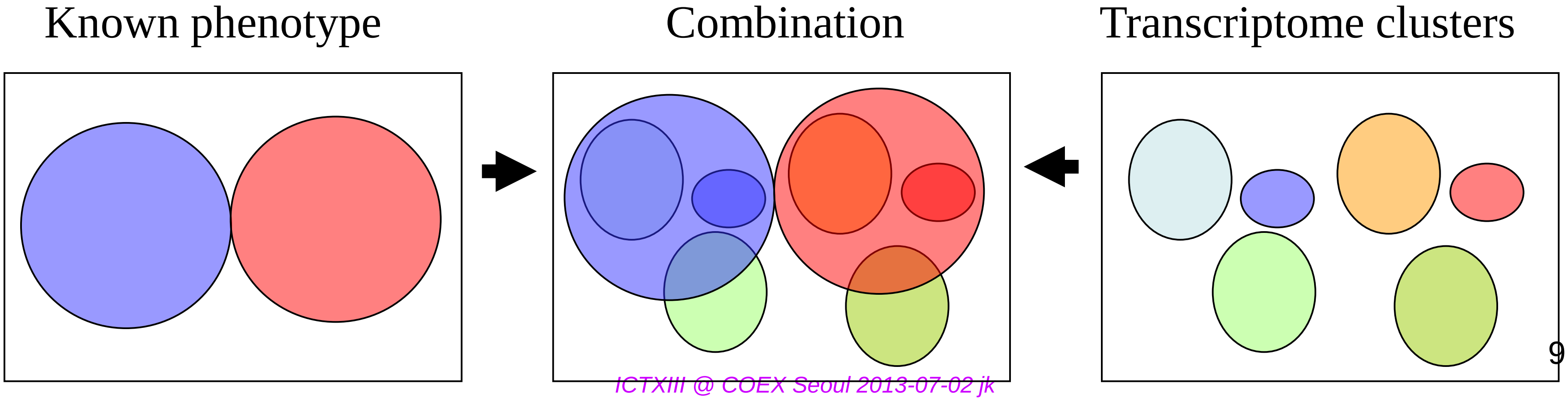
Comprehensive Monitoring by Microarray systems

Why “no phenotypic anchoring”

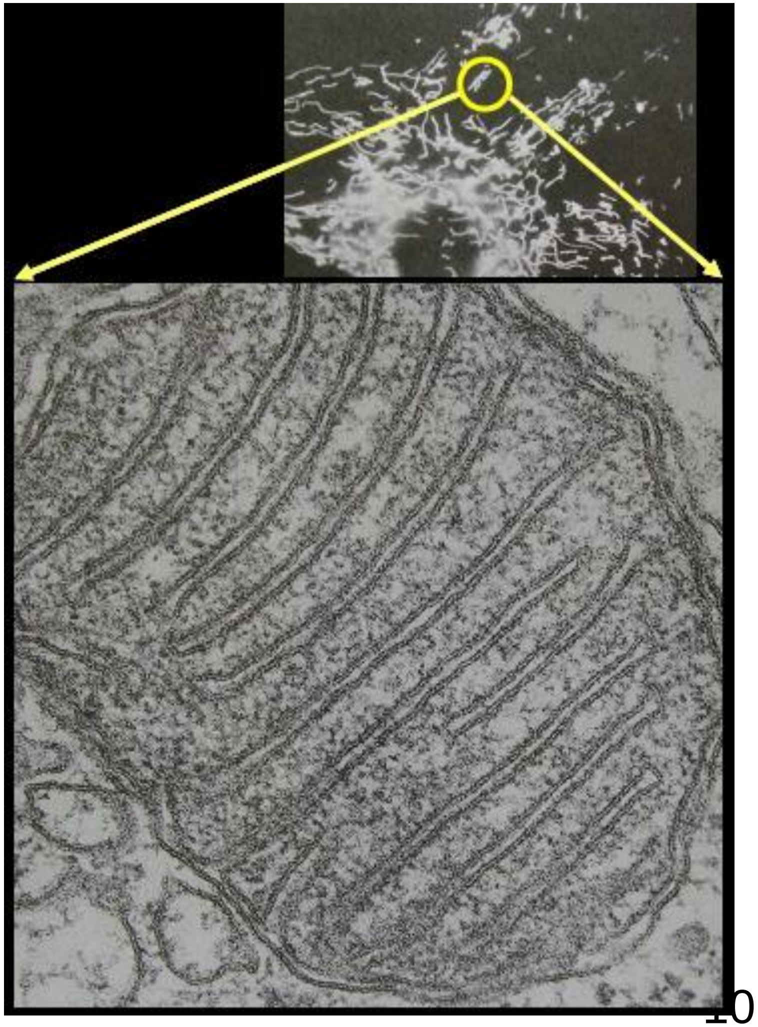
Why phenotype-independent

(metaphor)： Viral hepatitis

- Some time ago： HVA, B, and non-A non-B
  - Patients were divided into three categories
- Later：HVC appeared
  - The same Patient list were divided into four!
- Gene expression data (45,000)
  - If the genes are categorized by known factors, gene analysis will be limited by the factors.


Known phenotype
Combination
Transcriptome clusters

Toxicology by Transcriptomics Analogy….Electron microscopy



When Electron Microscopy was invented

Needed to write a new text book for practice

Needed to accumulate data

(even a new society)

….. it took 10~20 years to write text books

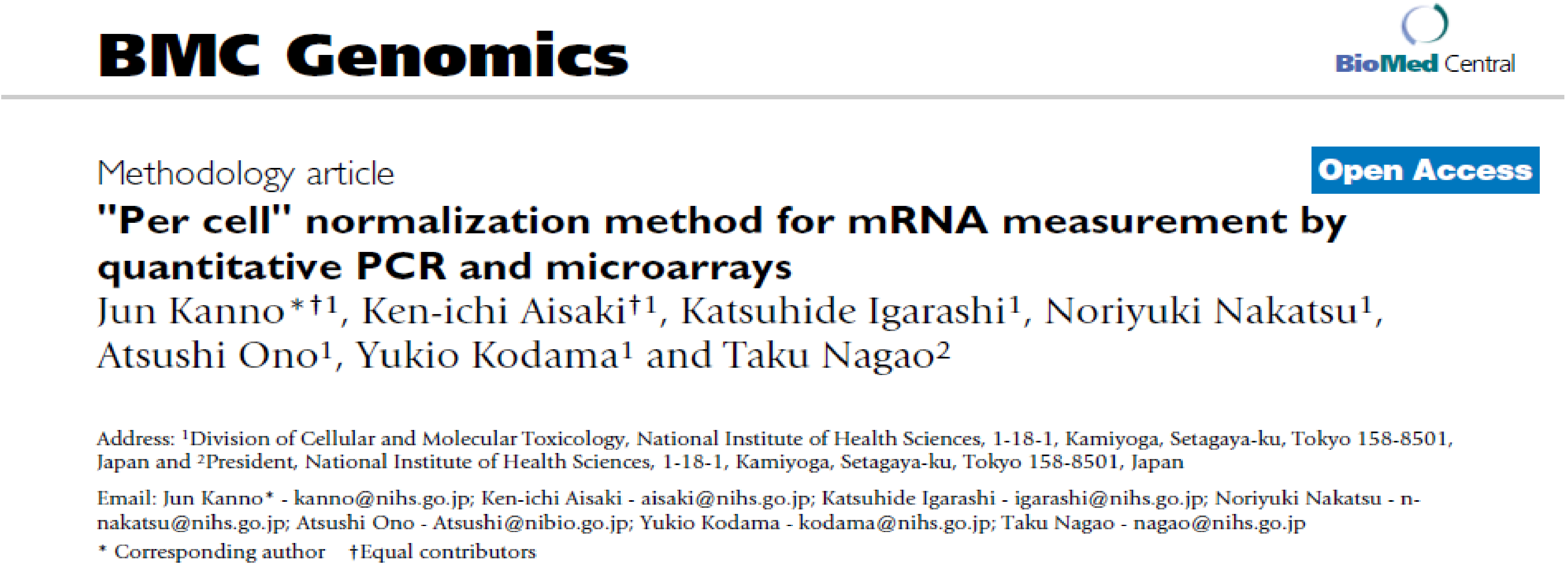
Toxicogenomics

Need to write a new text book for practice

Need to accumulate data

….. hopefully within 10 years !?

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

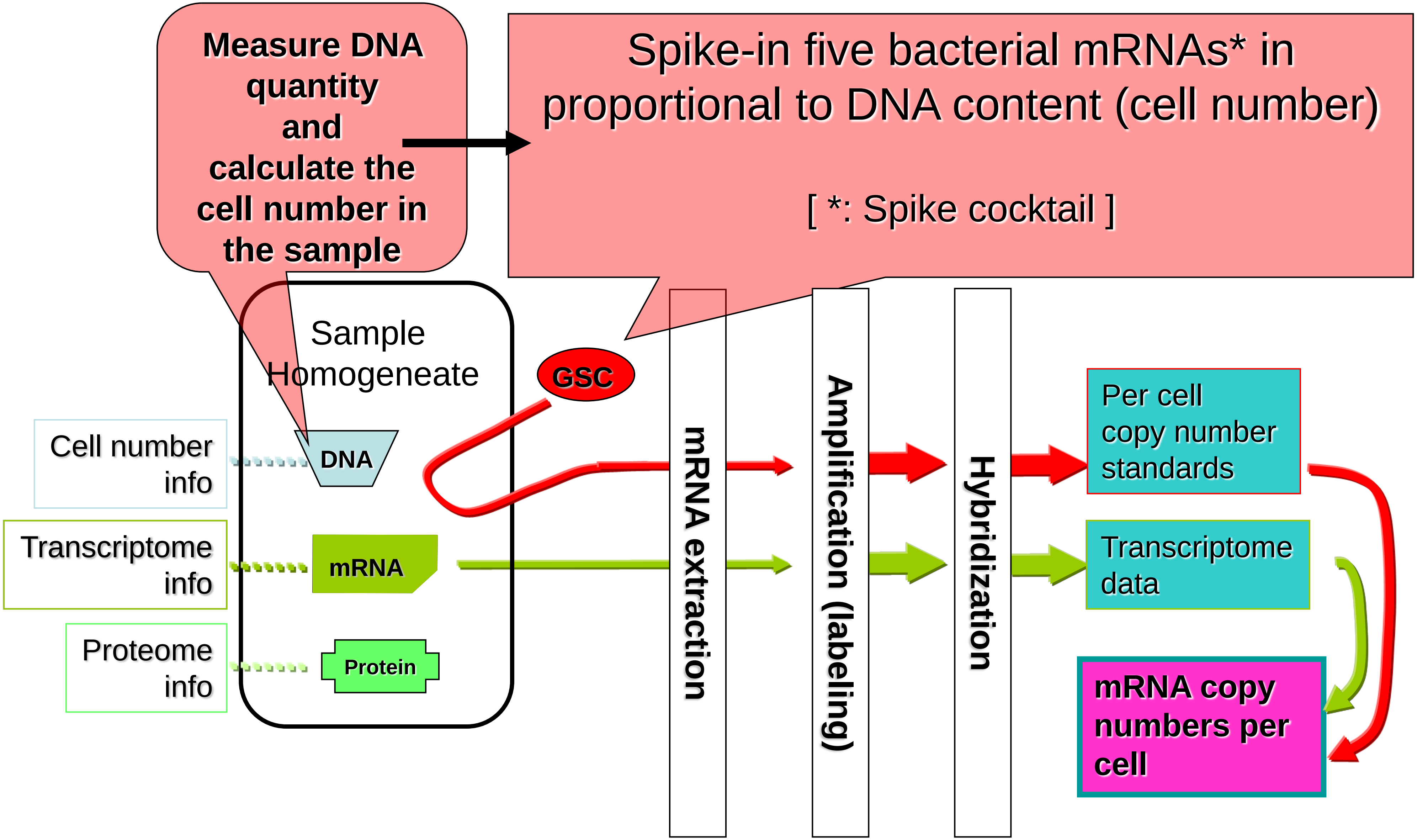
Jun Kanno*†[1], Ken-ichi Aisaki†[1], Katsuhide Igarashi[1], Noriyuki Nakatsu[1], Atsushi Ono[1], Yukio Kodama[1] and Taku Nagao[2]

Address: [1]Division of Cellular and Molecular Toxicology, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan and [2]President, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

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



Measure DNA quantity and calculate the cell number in the sample

Spike-in five bacterial mRNAs* in proportional to DNA content (cell number)

[*: Spike cocktail]

Sample Homogeneate

GSC

Cell number info

DNA

Transcriptome info

mRNA

Proteome info

Protein

mRNA extraction

Amplification (labeling)

Hybridization

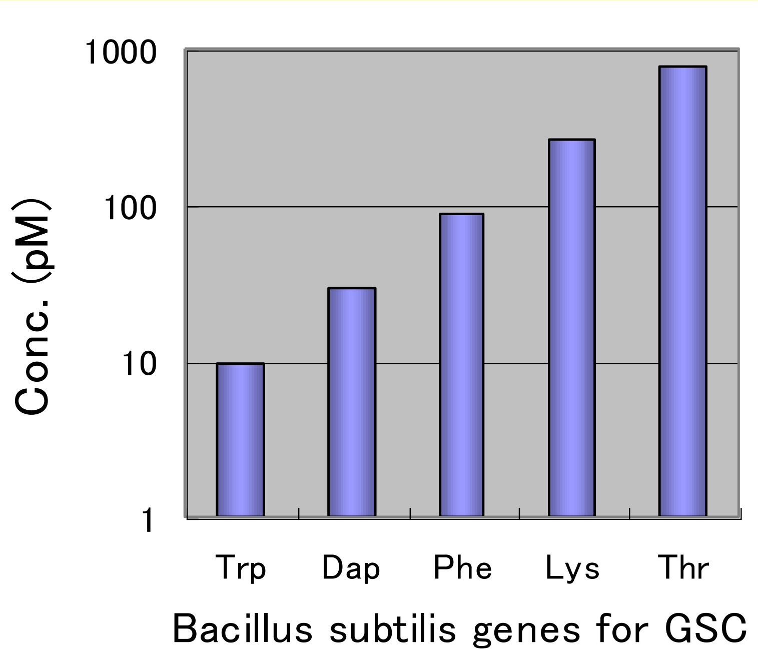
Per cell copy number standards

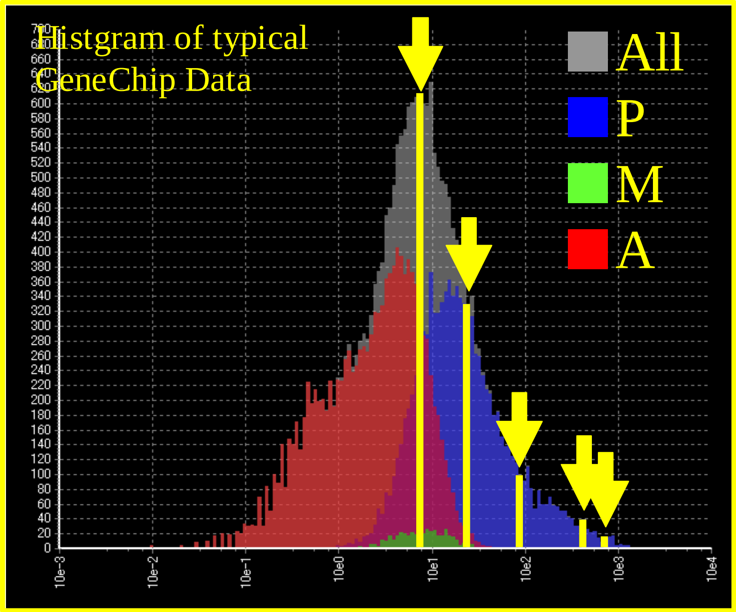
Transcriptome data

mRNA copy numbers per cell

* Grade-dosed Spike Cocktail (GSC)

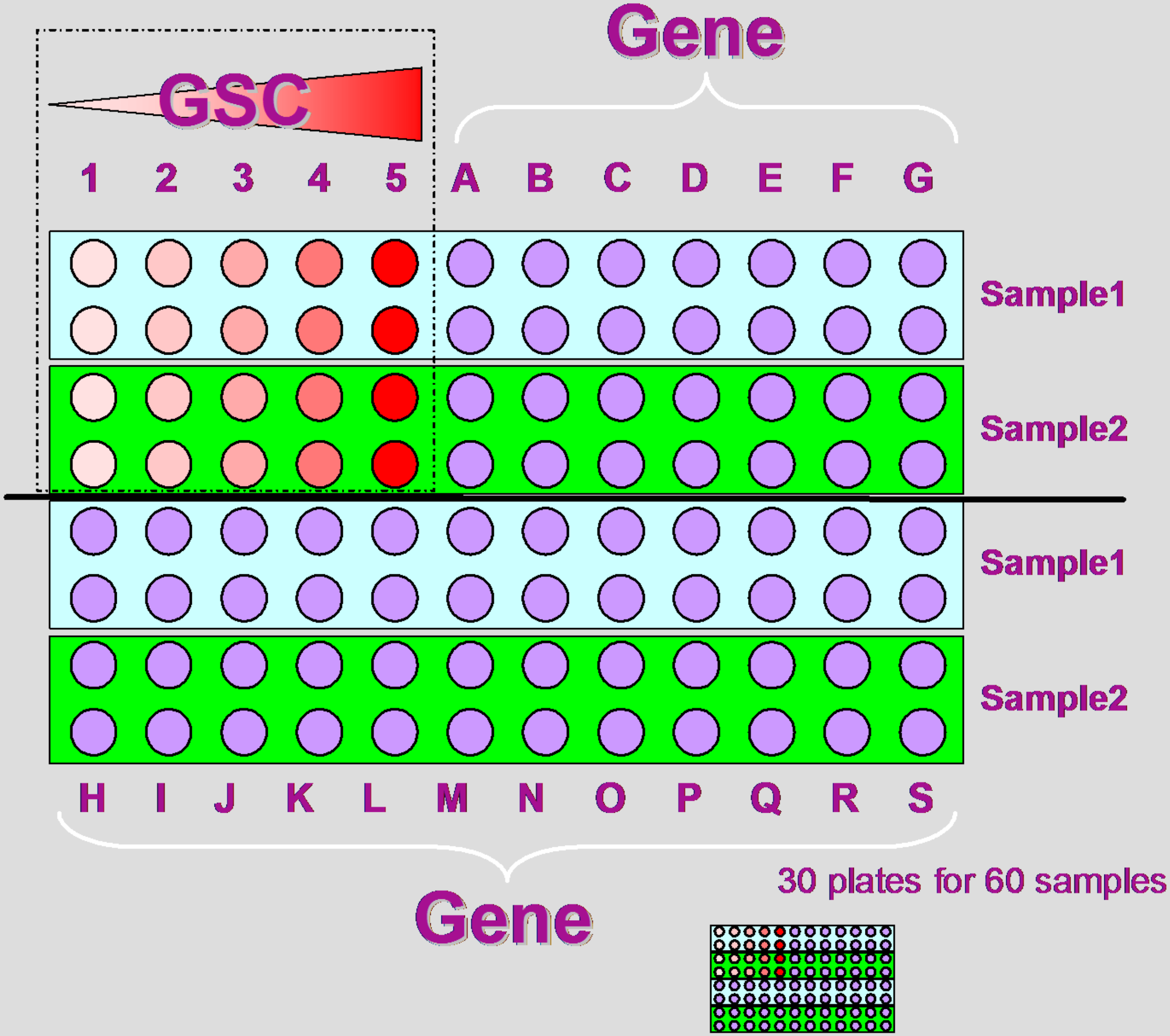
- Spike mRNAs that do not cross-hybridize with sample mRNA
- Affymetrix “Present” call data are covered by GSC range





Percellome Quantitative-PCR

(by ABI PRISM 7900HT / SYBR Green)

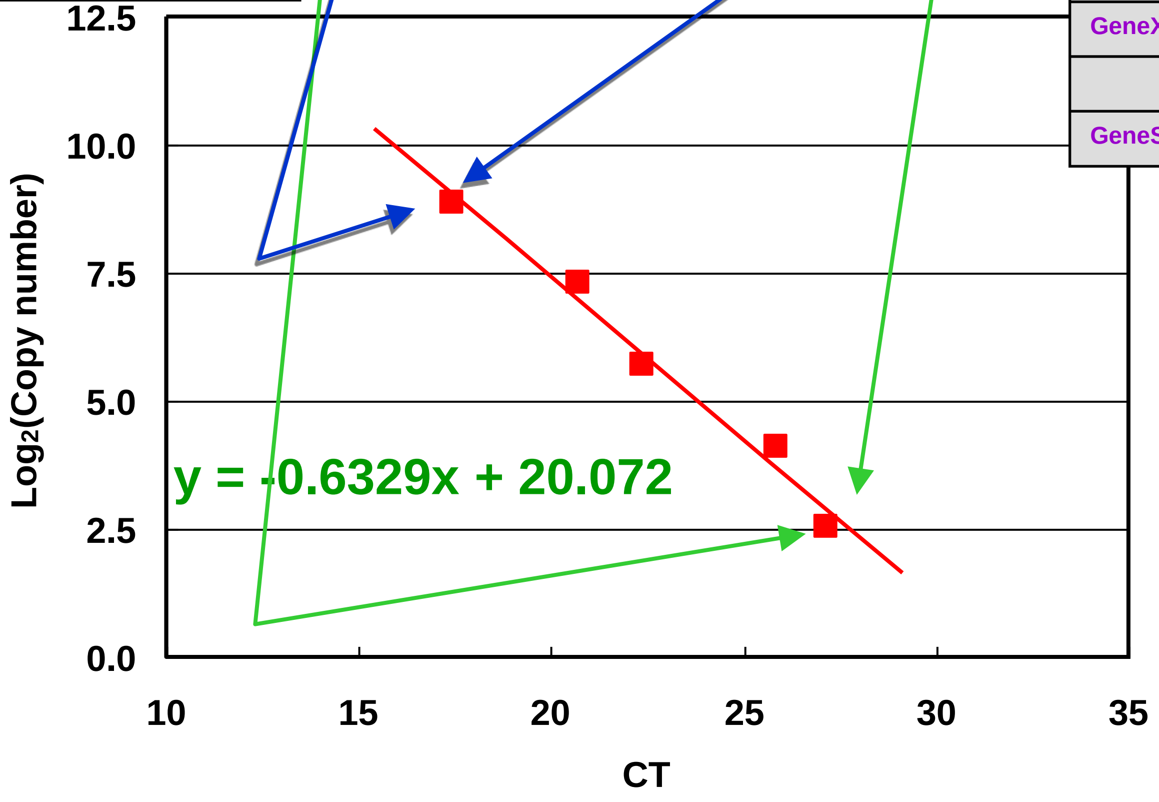

Gene
GSC
1
2
3
4
5
A
B
C
D
E
F
G
Sample1
Sample2
Sample1
Sample2
H
I
J
K
L
M
N
O
P
Q
R
S
Gene

30 plates for 60 samples

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

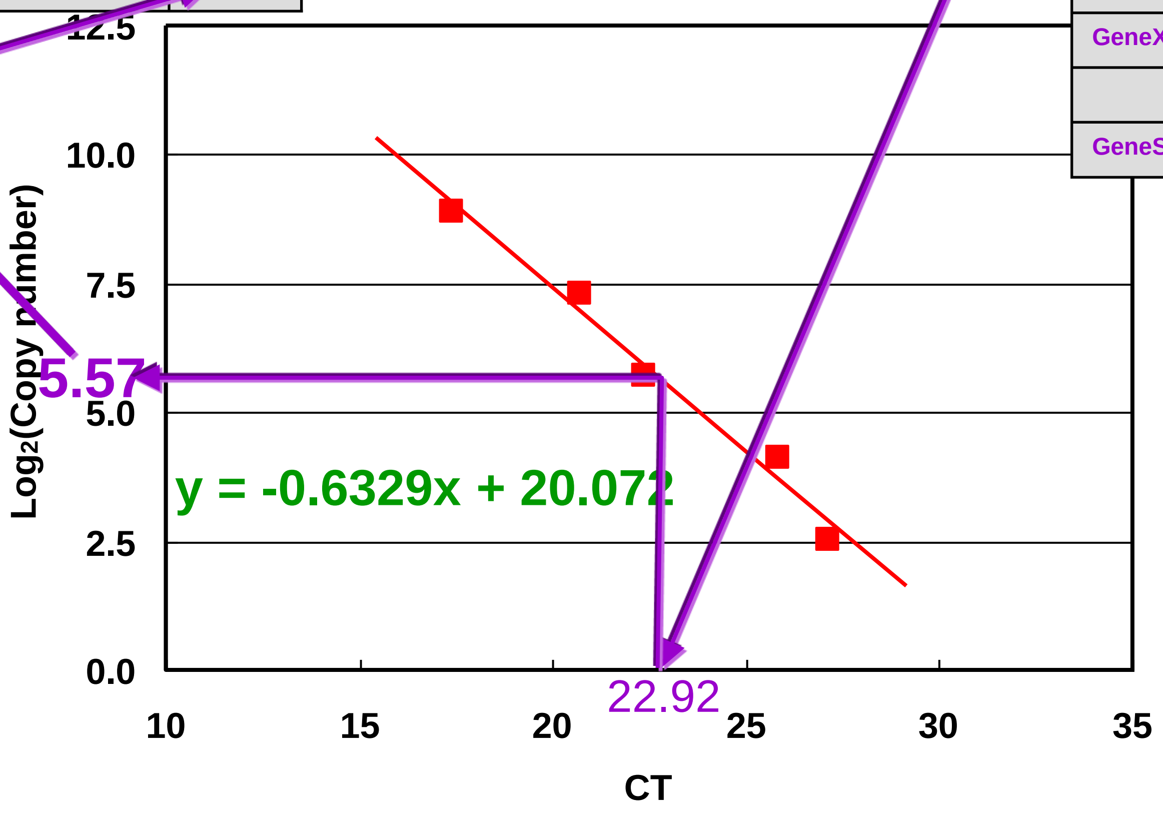


Percellome Q-PCR normalization

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	**47.5**

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

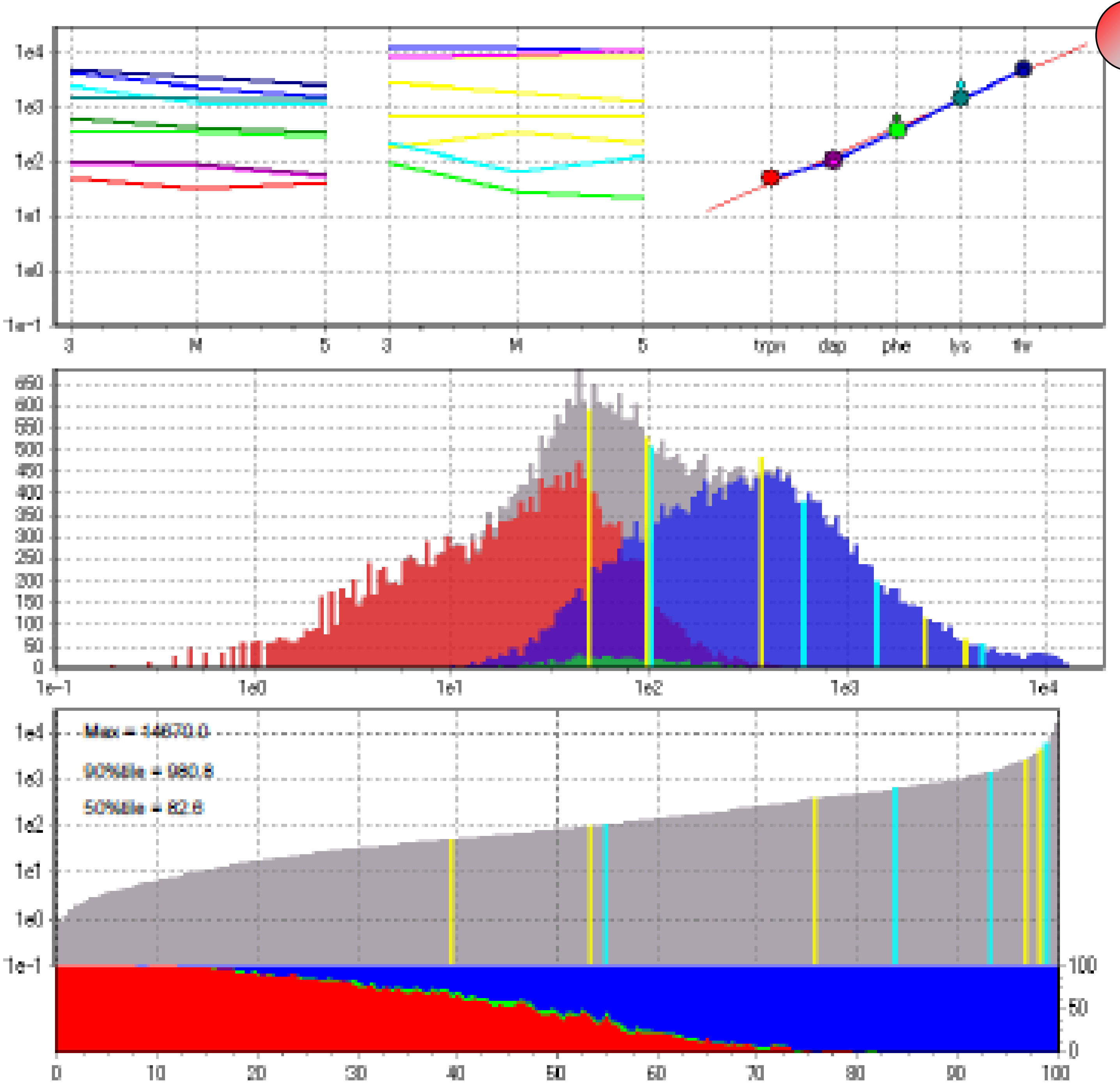
$2^{5.57}$=**47.5**



SCal4

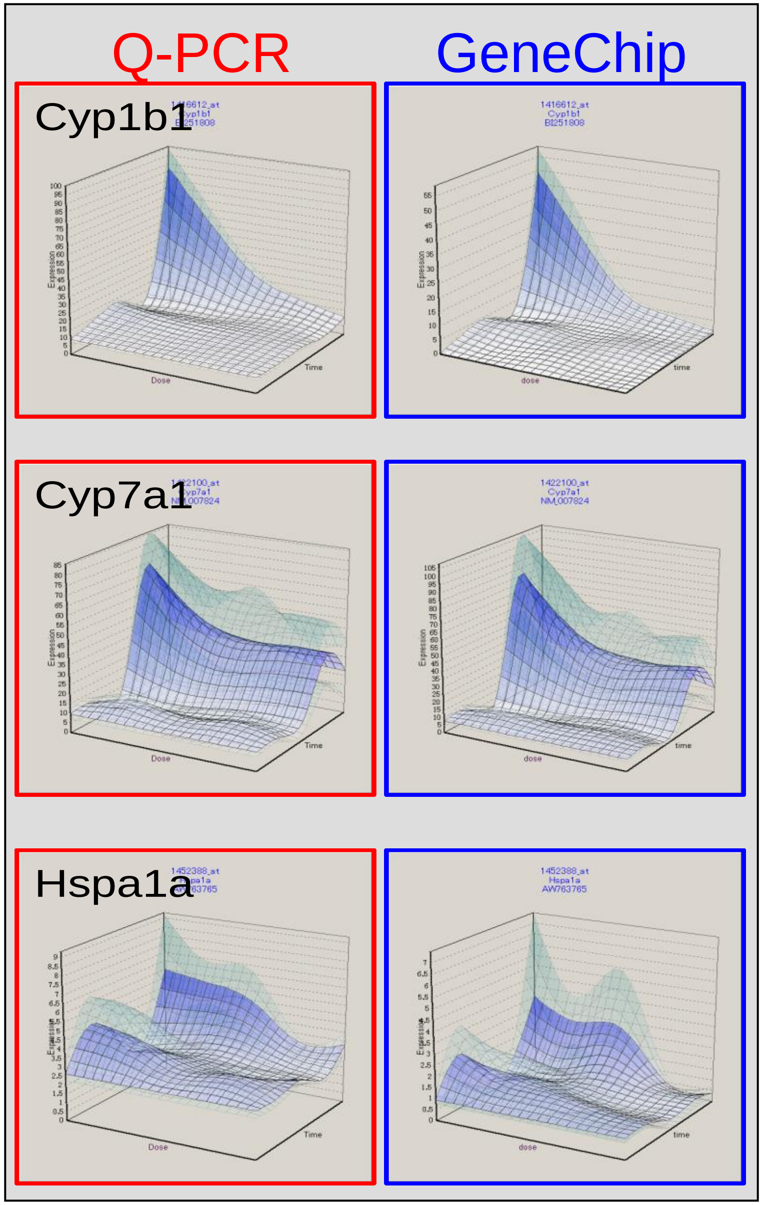
Affymetrix GeneChip (Mouse 430 v2 Rat, Human, Xenopus)

Exon Array (human and mouse) now under trial

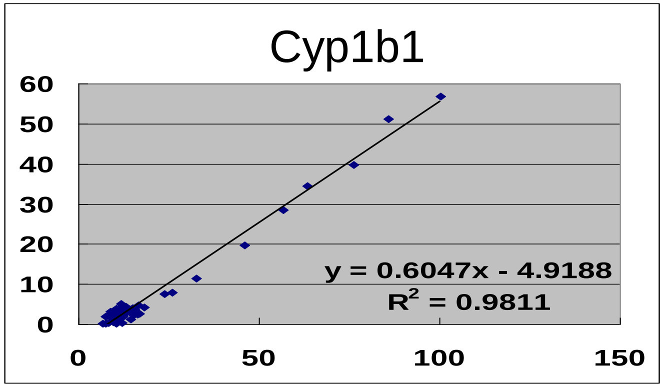


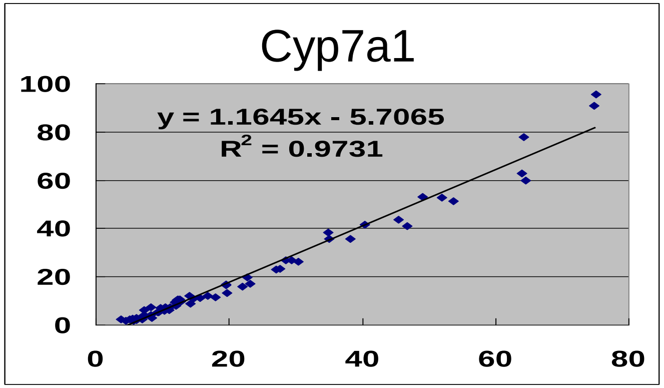
Percellome Q-PCR versus GeneChip

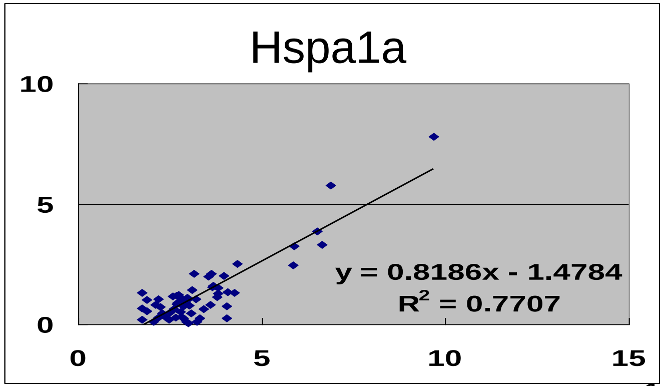
TCDD /// 5x4 n=3, 60 sample /// GSC(5)+19 primer pair set1



Copy number by GeneChip



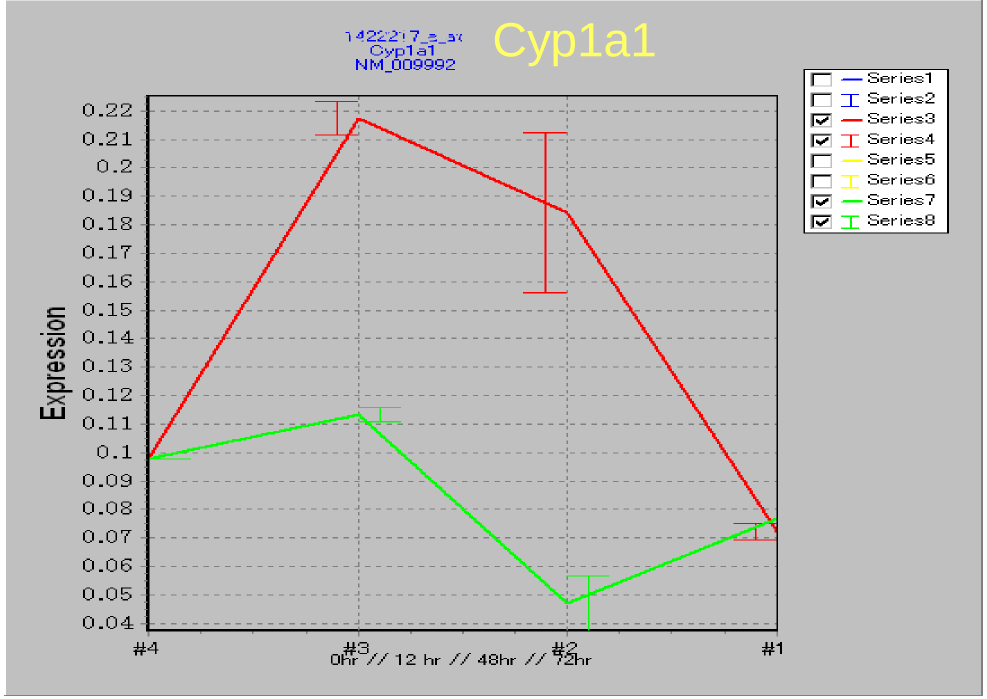




Copy number by Q-PCR

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

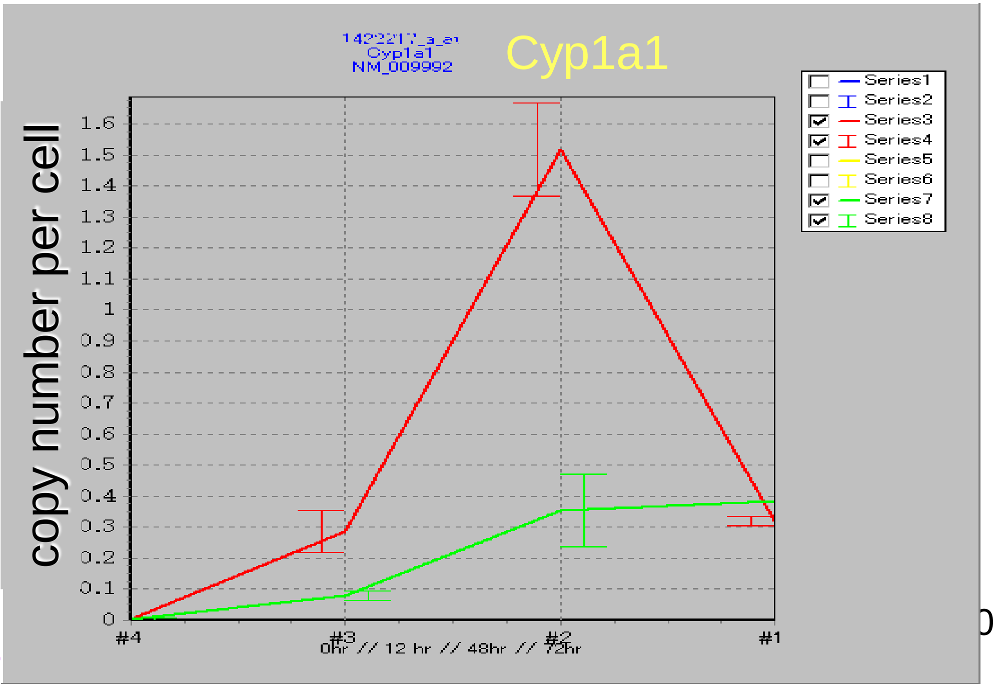
Global Normalization



In vitro Experiment

Cyp1a1 induction by an AhR ligand chemical

Percellome

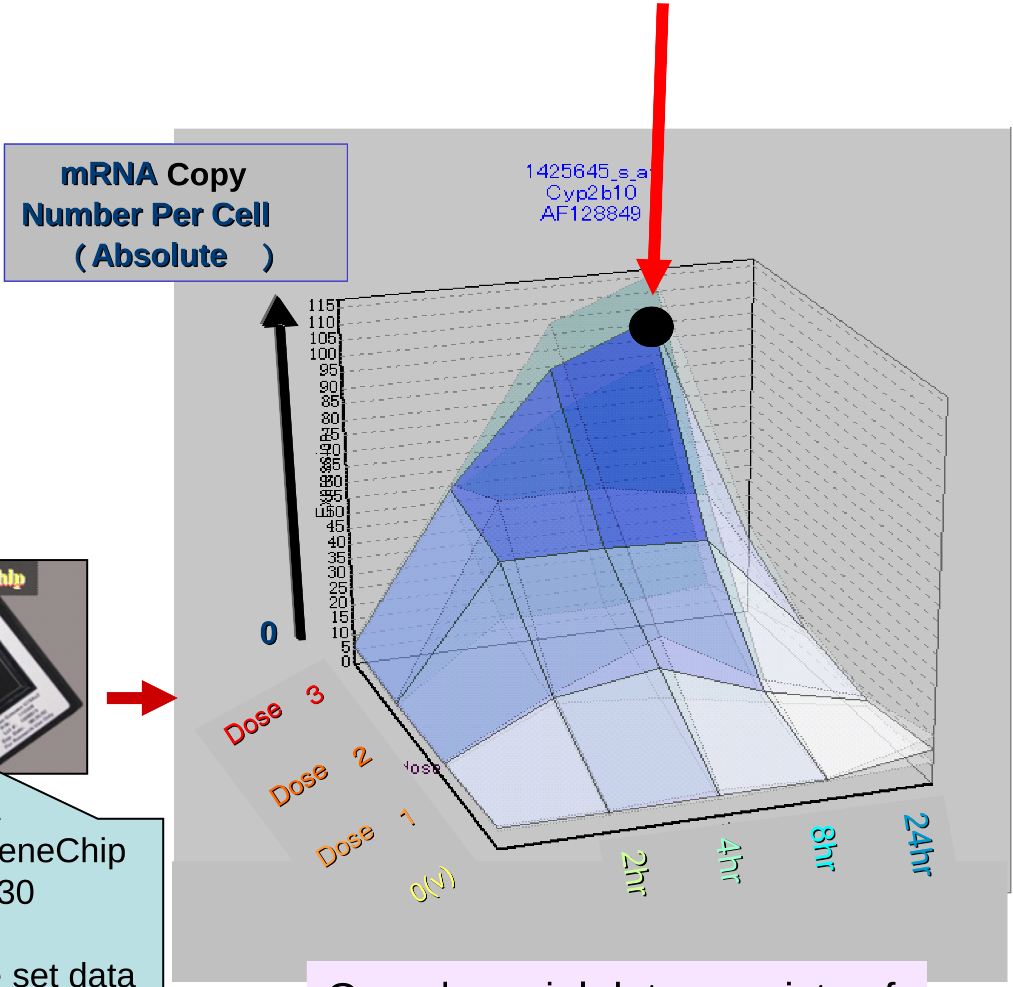


Standard Protocol of Percellome Project

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)

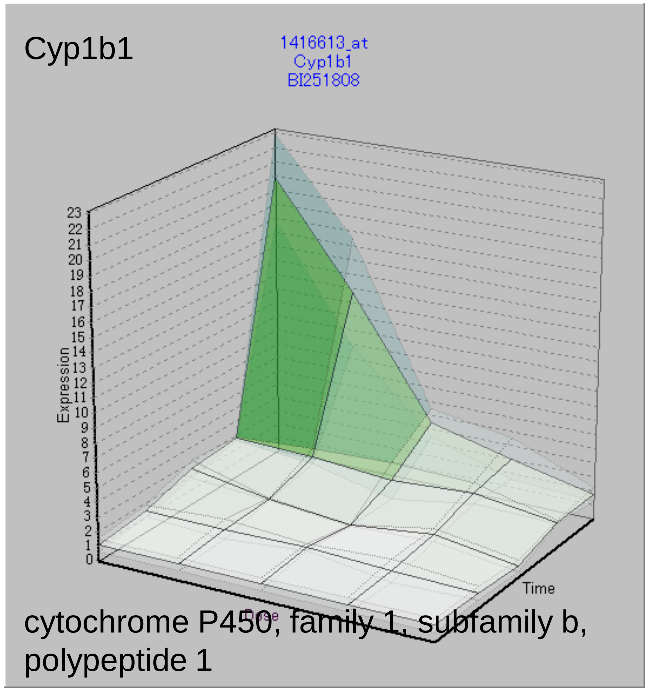
Affymetrix GeneChip
MOE430

45,000 probe set data

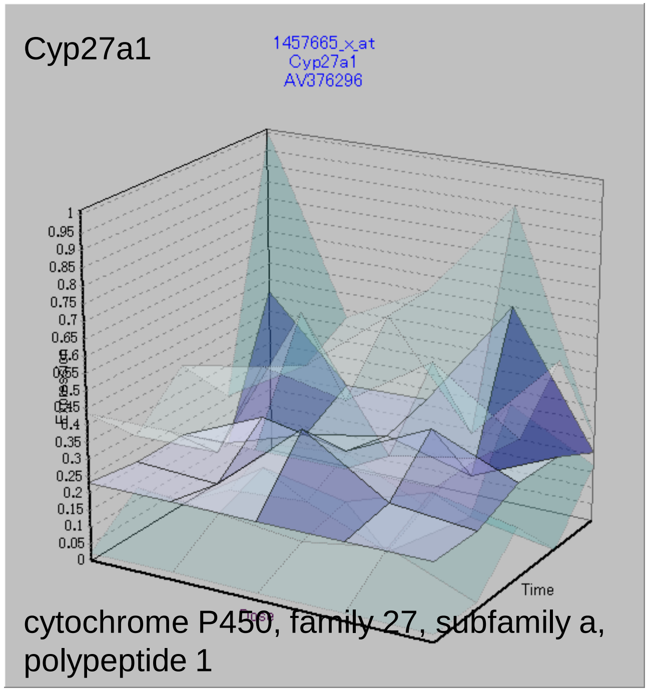
One chemcial data consists of
45,000 layers of 3D surface

ICTXIII @ COEX Seoul 2013-07-02 jk

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


Cyp1b1

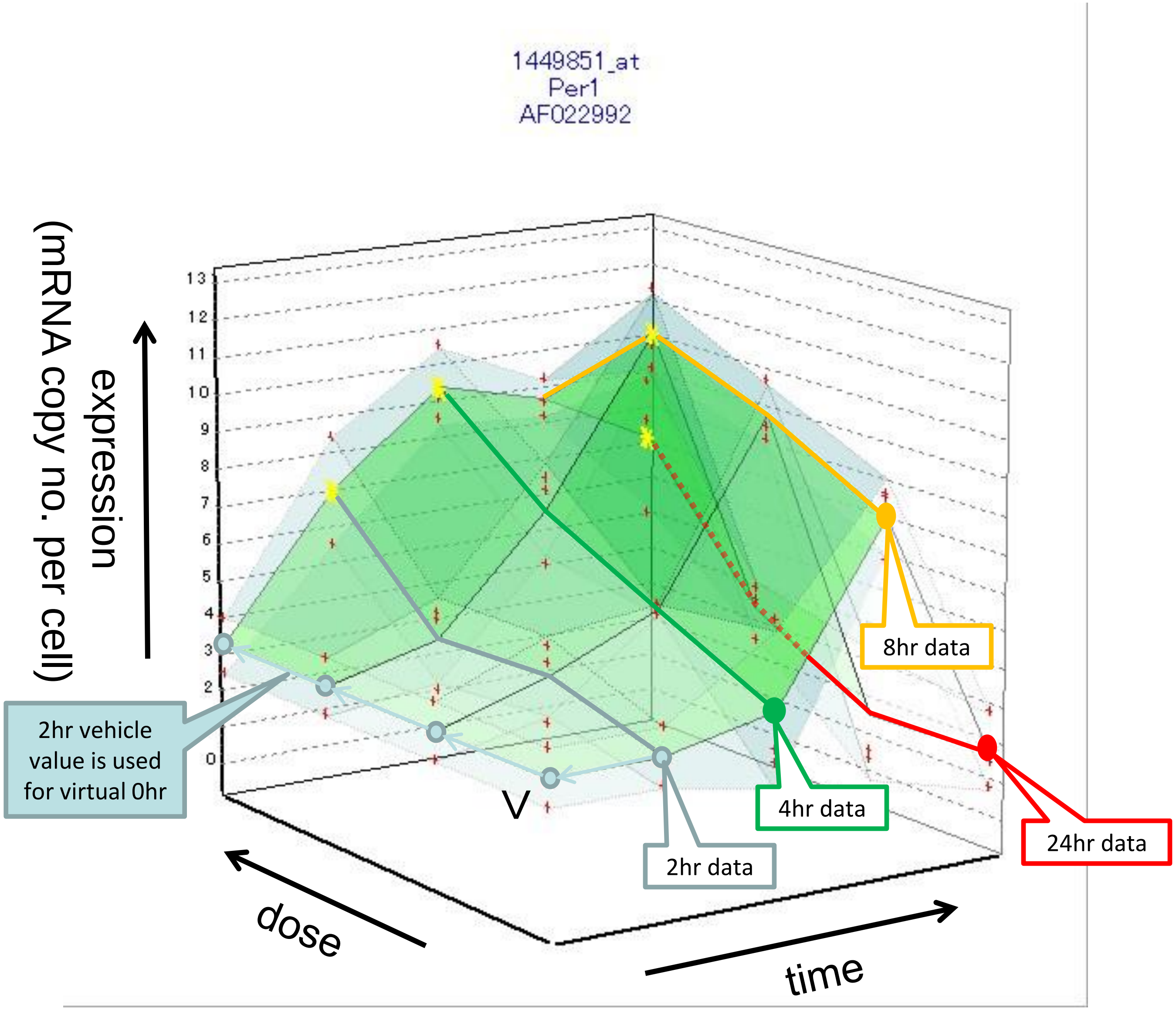
cytochrome P450, family 1, subfamily b, polypeptide 1


Cyp27a1

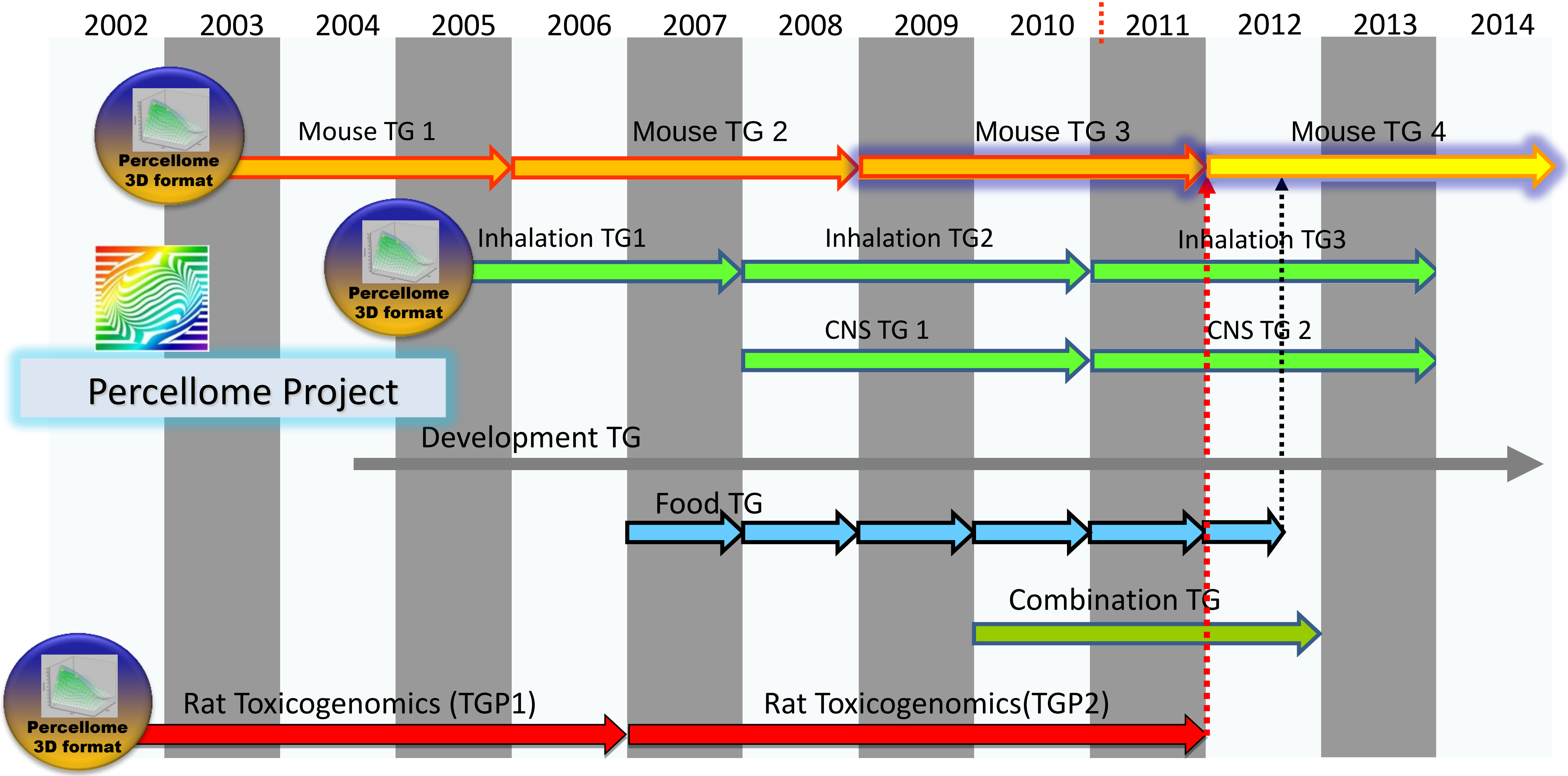
cytochrome P450, family 27, subfamily a, polypeptide 1

ICTXIII @ COEX Seoul 2013-07-02 jk

Figure 1


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

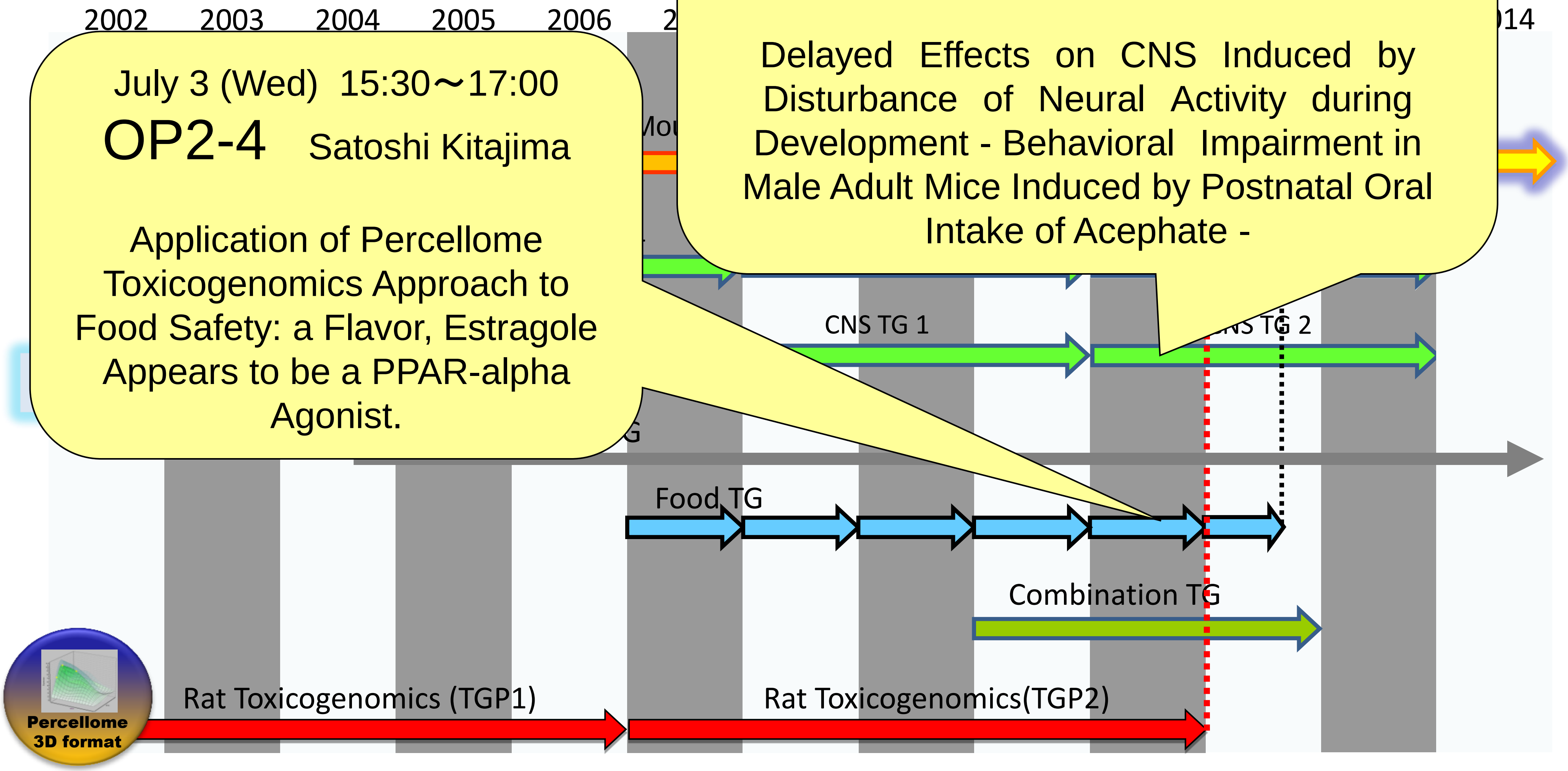
Percellome TG Project / Div Cell&Mol Tox, NIHS


2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
Percellome 3D format
Mouse TG 1
Mouse TG 2
Mouse TG 3
Mouse TG 4
Percellome 3D format
Inhalation TG1
Inhalation TG2
Inhalation TG3
CNS TG 1
CNS TG 2
Percellome Project
Development TG
Food TG
Combination TG
Percellome 3D format
Rat Toxicogenomics (TGP1)
Rat Toxicogenomics(TGP2)

All of these data are Percellome data

ICTXIII @ COEX Seoul 2013-07-02 jk

Percellome TG Pr


2002
2003
2004
2005
2006
2
014
Mou
CNS TG 1
NS TG 2
G
Food TG
Combination TG
Percellome 3D format
Rat Toxicogenomics (TGP1)
Rat Toxicogenomics(TGP2)

July 3 (Wed) 10:30～12:00

S18-5 Kentaro Tanemura

Delayed Effects on CNS Induced by Disturbance of Neural Activity during Development - Behavioral Impairment in Male Adult Mice Induced by Postnatal Oral Intake of Acephate -

July 3 (Wed) 15:30～17:00

OP2-4 Satoshi Kitajima

Application of Percellome Toxicogenomics Approach to Food Safety: a Flavor, Estragole Appears to be a PPAR-alpha Agonist.

All of these data are Percellome data

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
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
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
oxin)
)
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

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

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!

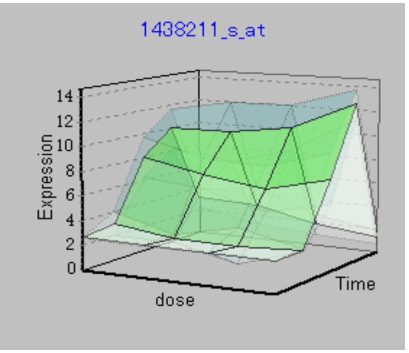
- Top dose:
  - – Default setting
    - dose that does not induce macroscopic and microscopic changes in 24 hours after single dose.
  - – Hormones or hormone-like chemicals (nuclear receptor ligands)
    - dose that gives near maximum response (ED70~80) (referring to binding assay or reporter gene assay data)

- Middle and Low dose:
  - – √10 ratio (10, 3, 1 or 20, 7, 2)

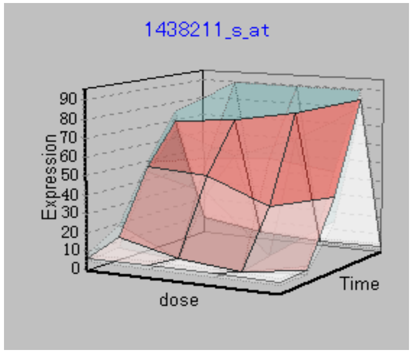
Circadian change is significant in almost all organs

CCl_4 Single oral exposure

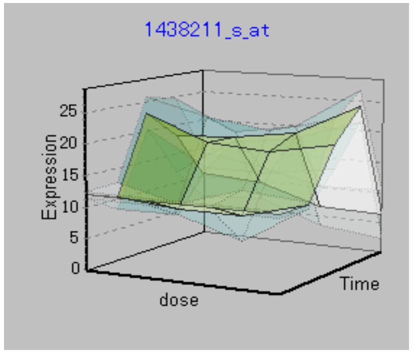
Lung



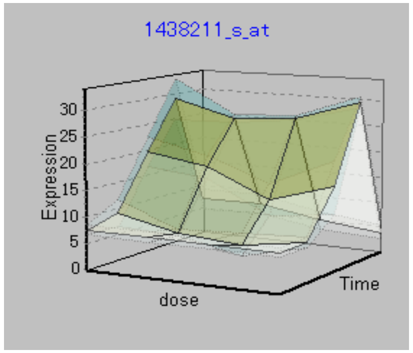
Liver



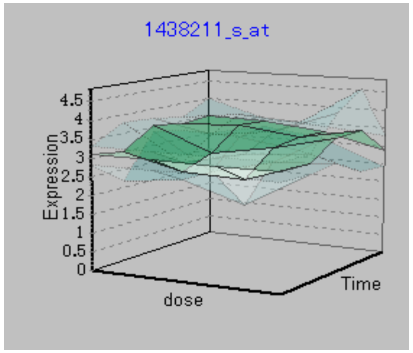
Kidney



Heart



Testis



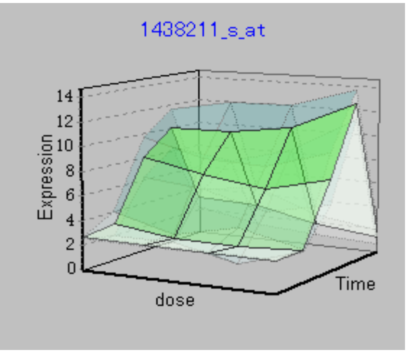
Dbp (circadian gene)

D site albumin promoter binding protein

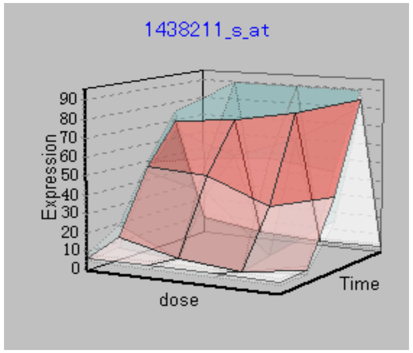
Circadian change is significant in almost all organs

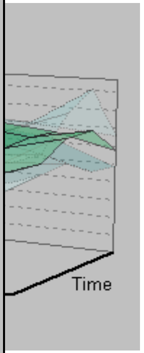
CCl$_4$ Single or

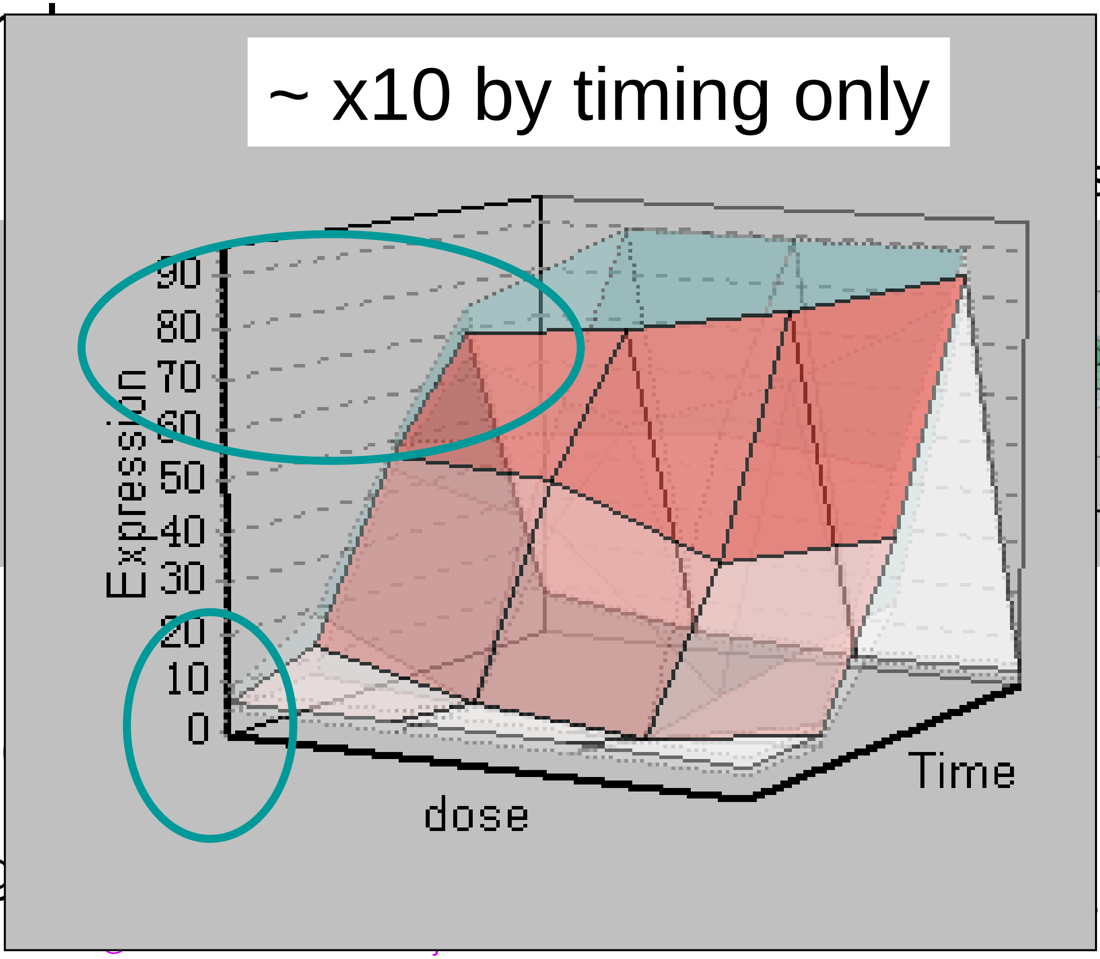
Lung



Liver







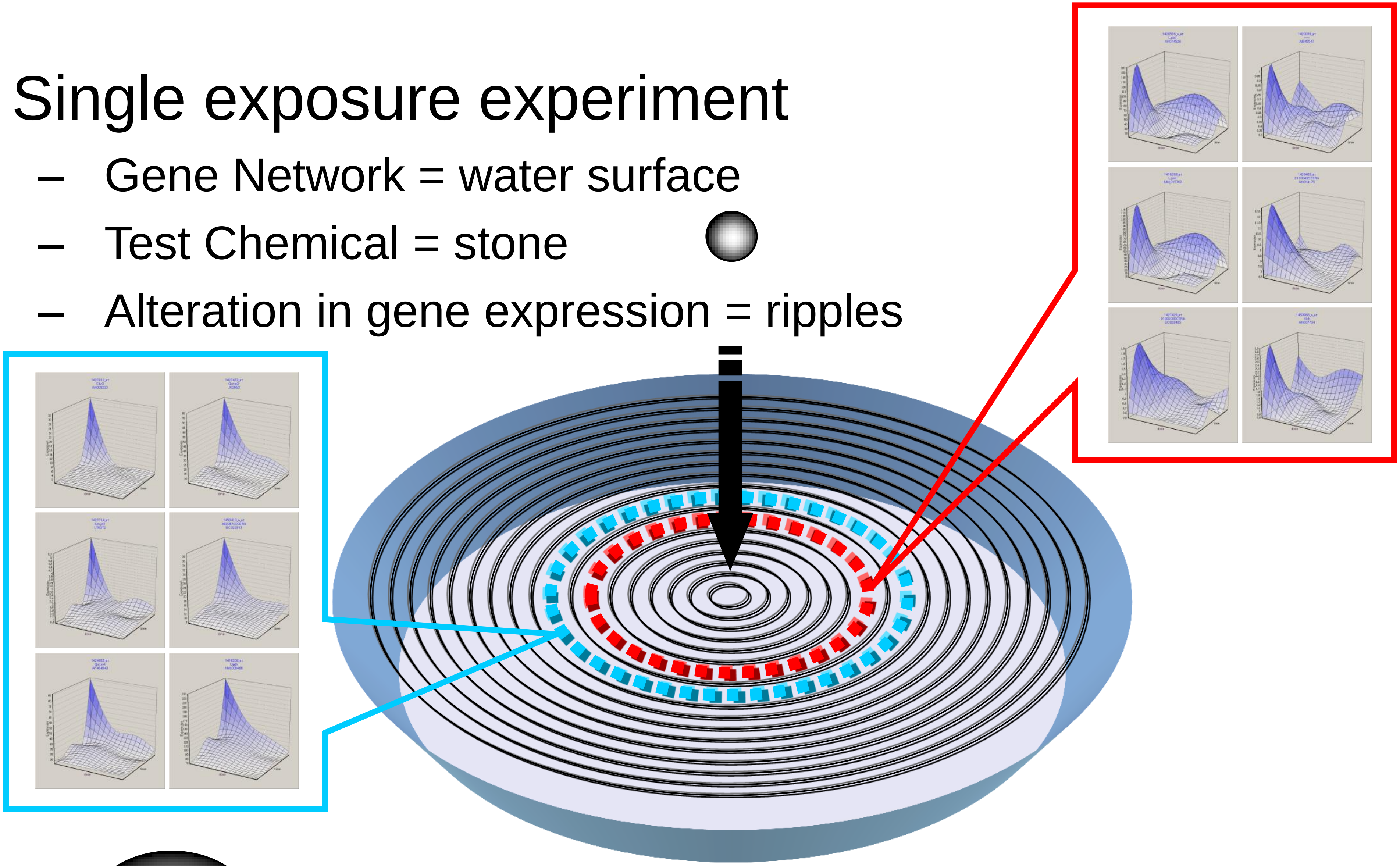
~ x10 by timing only

Dbp (circadian g

D site albumin promoter binding

- Percellome data analysis strategy
  - Comprehensive
  - Unsupervised

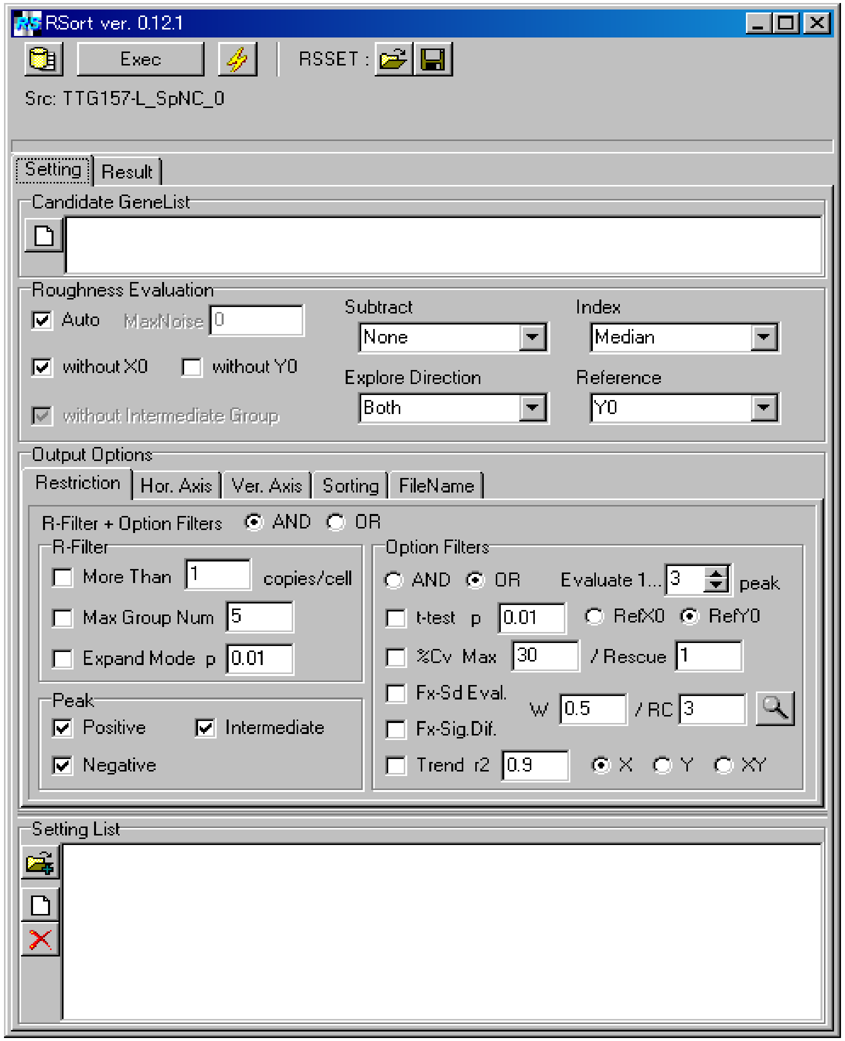
- Single exposure experiment
  - Gene Network = water surface
  - Test Chemical = stone
  - Alteration in gene expression = ripples



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

RSort Program by Ken-ichi Aisaki

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



RSort ver. 0.12.1

Src: TTG157-L_SpNC_0

Setting | Result

Candidate GeneList

Roughness Evaluation

☑ Auto MaxNoise 0

☑ without X0 ☐ without Y0

☑ without Intermediate Group

Subtract: None

Index: Median

Explore Direction: Both

Reference: Y0

Output Options

Restriction | Hor. Axis | Ver. Axis | Sorting | FileName

R-Filter + Option Filters ● AND ○ OR

R-Filter

☐ More Than 1 copies/cell

☐ Max Group Num 5

☐ Expand Mode p 0.01

Peak

☑ Positive ☑ Intermediate

☑ Negative

Option Filters

○ AND ● OR Evaluate 1... 3 peak

☐ t-test p 0.01 ○ RefX0 ● RefY0

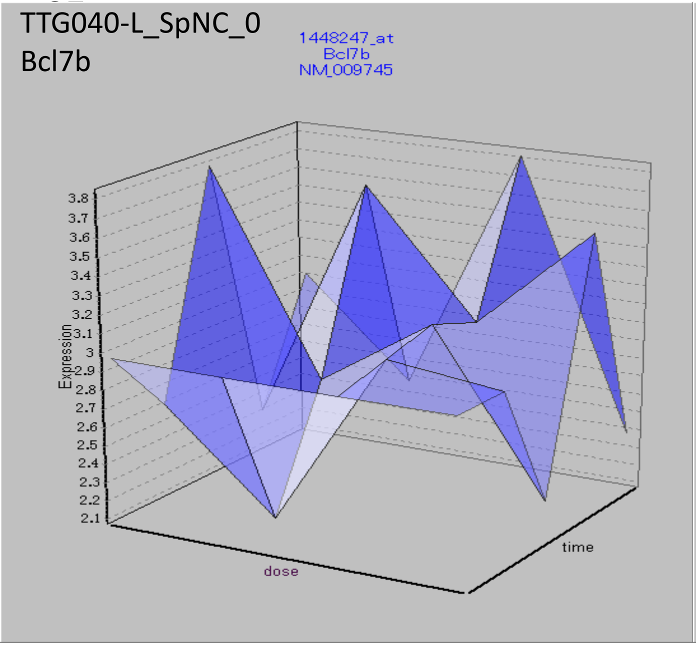
☐ %Cv Max 30 / Rescue 1

☐ Fx-Sd Eval. W 0.5 / RC 3

☐ Fx-Sig.Dif.

☐ Trend r2 0.9 ● X ○ Y ○ XY

Setting List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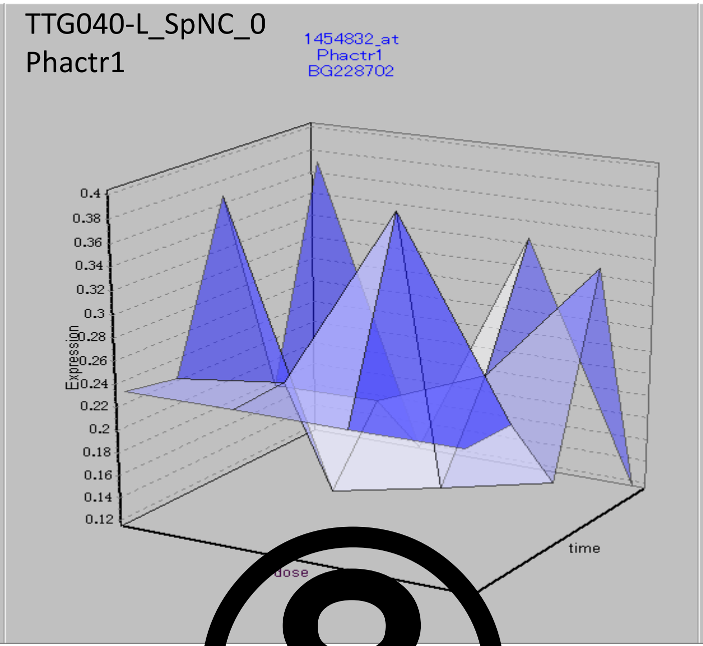
Bcl7b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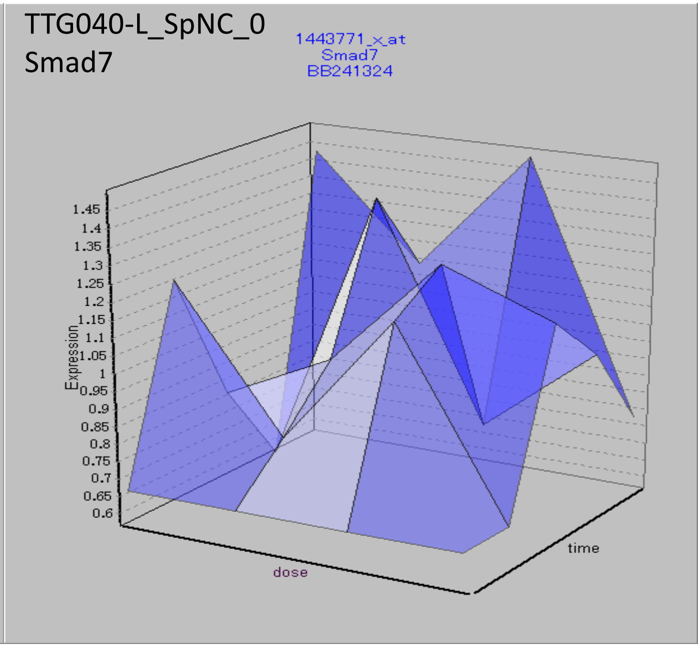
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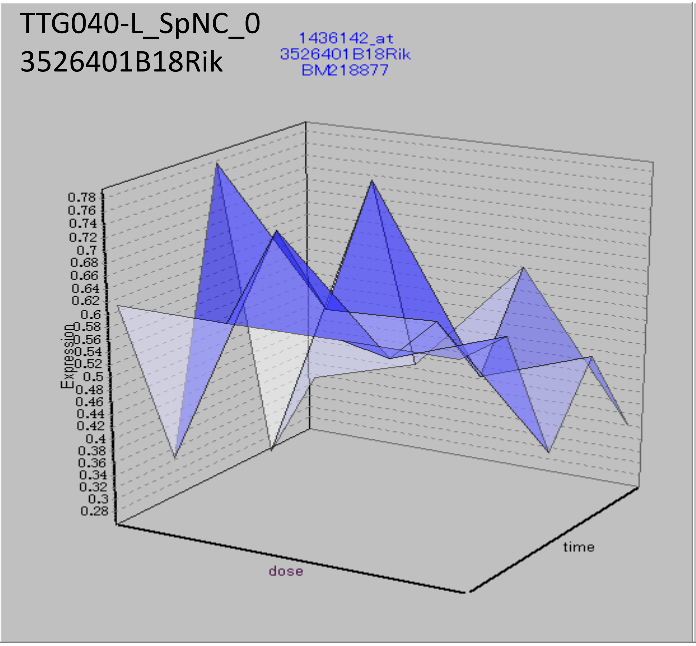
Smad7

⑧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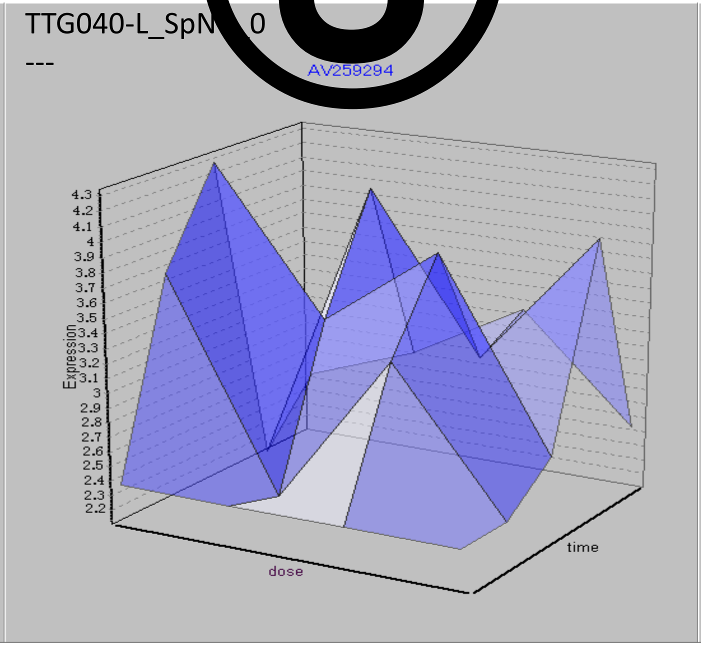
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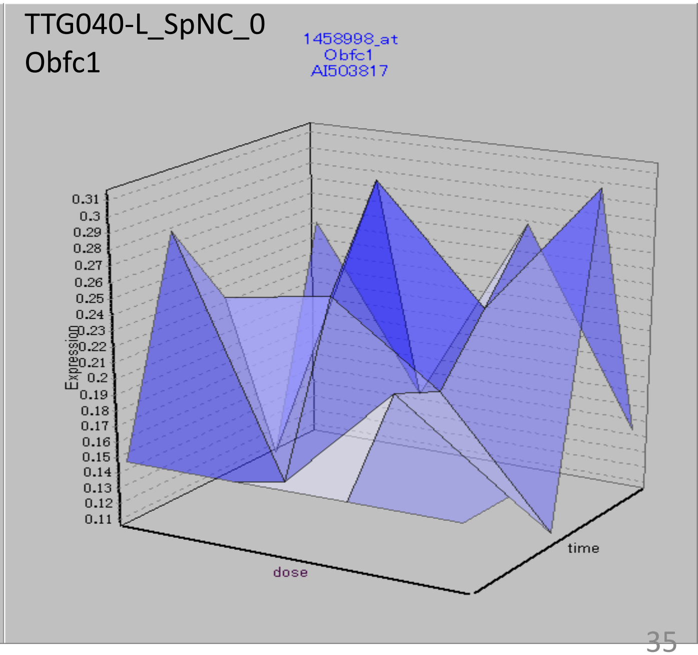
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Obfc1

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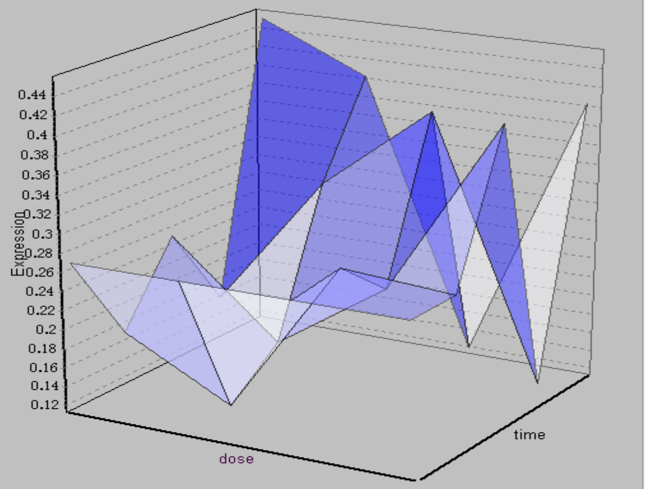

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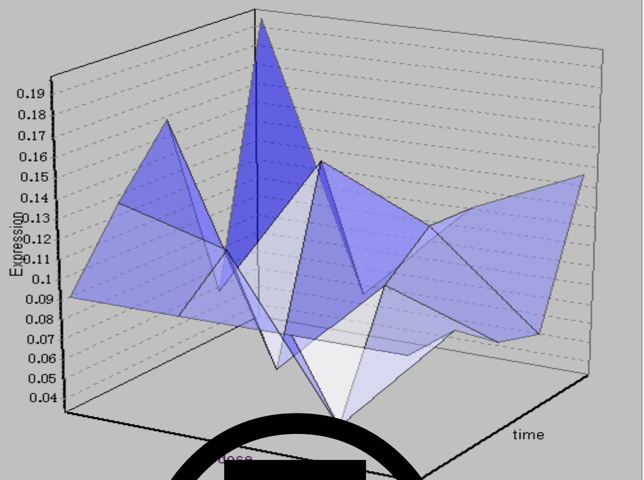

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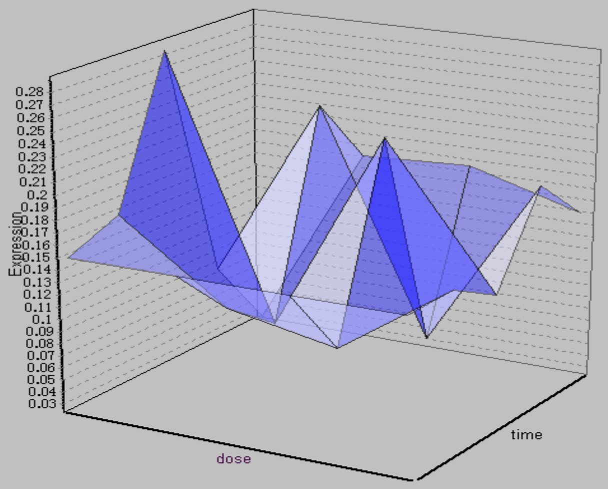
Aqp6



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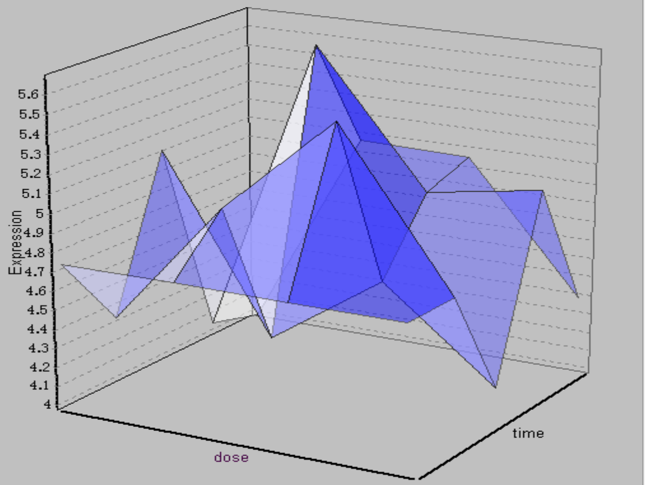
Rgl2



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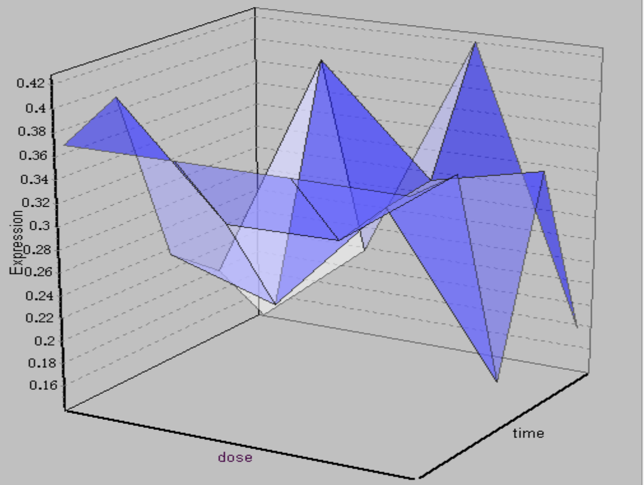
D10Ertd755e



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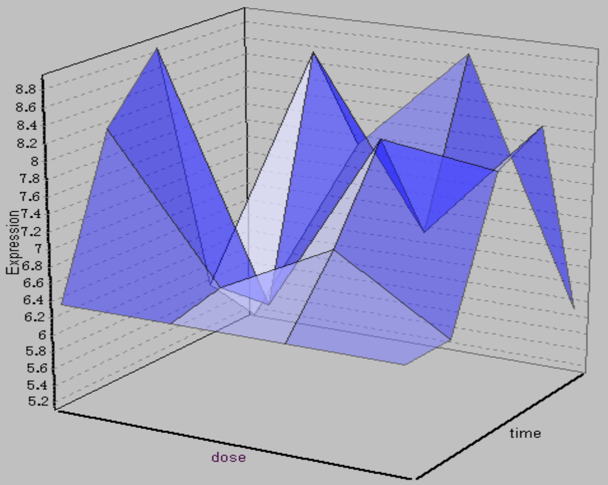

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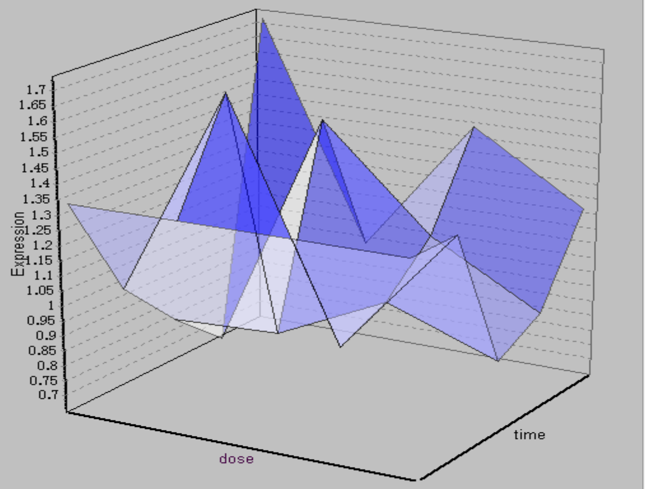
Mthfr



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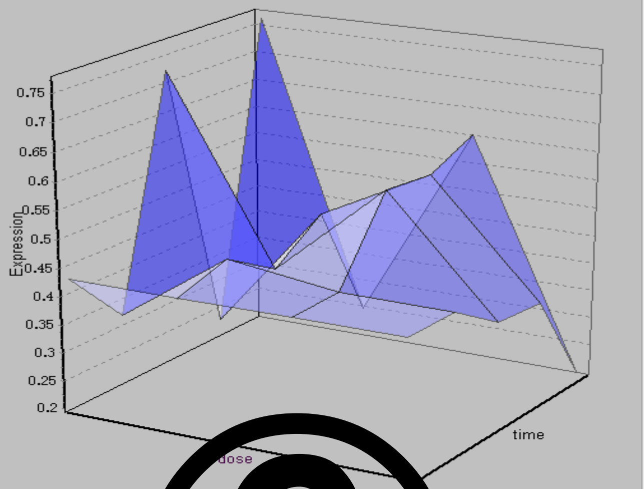
Gria1



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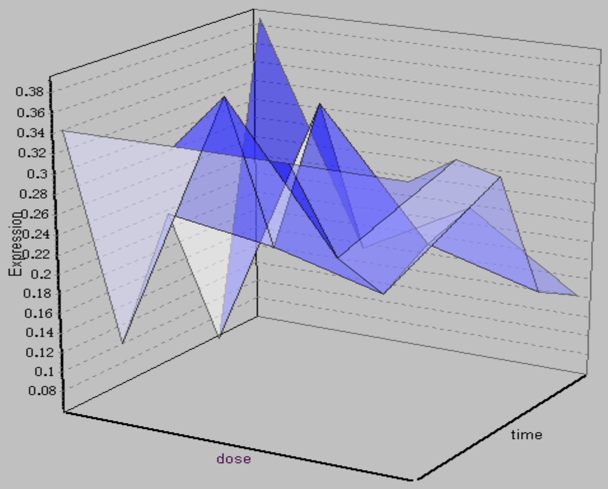

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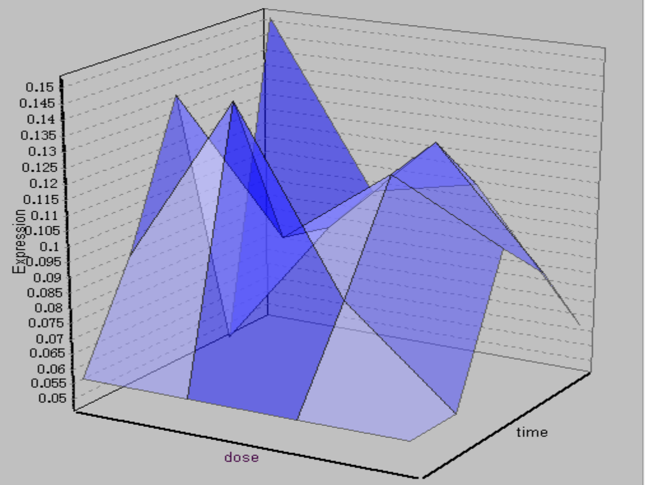
TTG040-L_SpNC_0
Agrn



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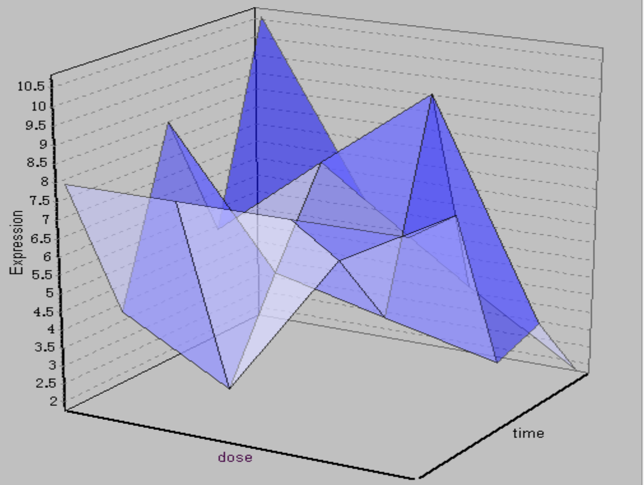

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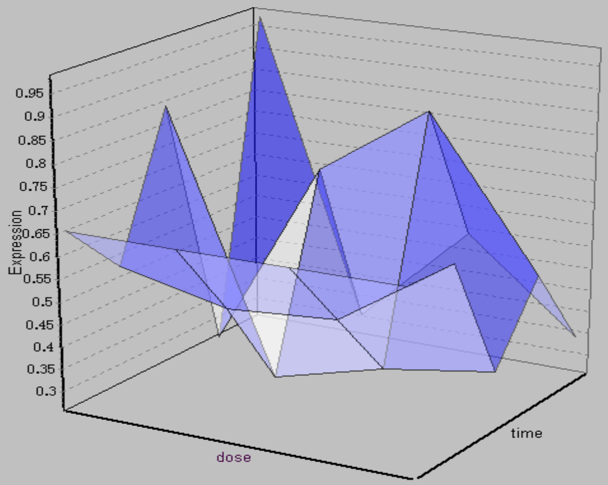
Fcrl1



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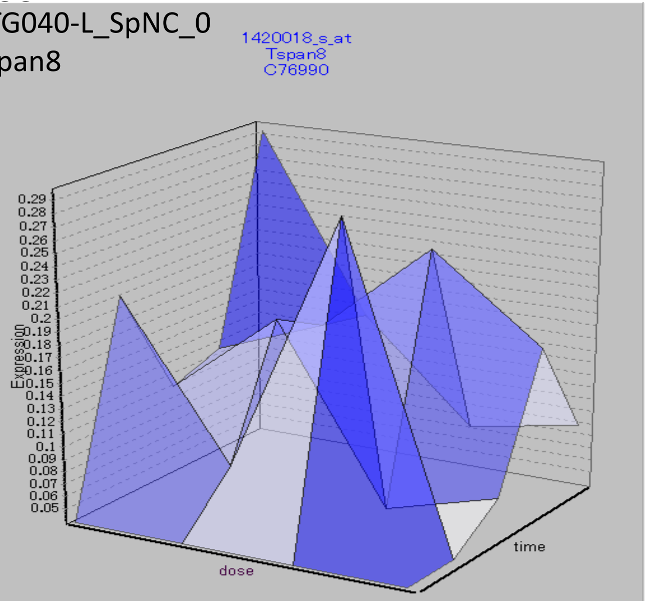

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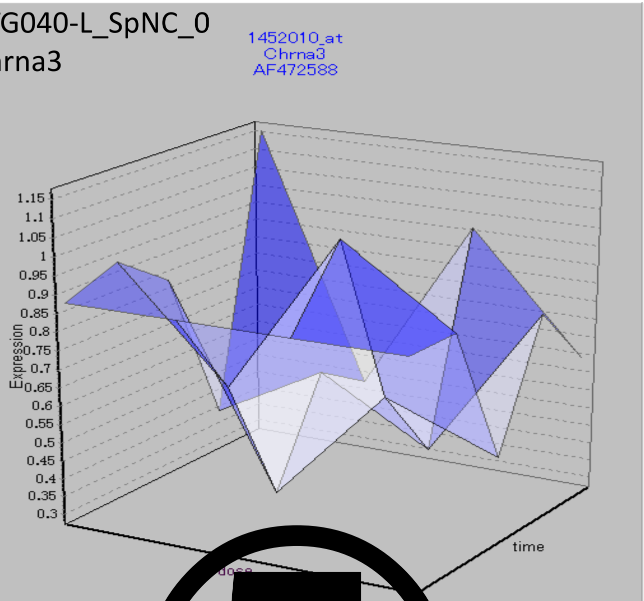

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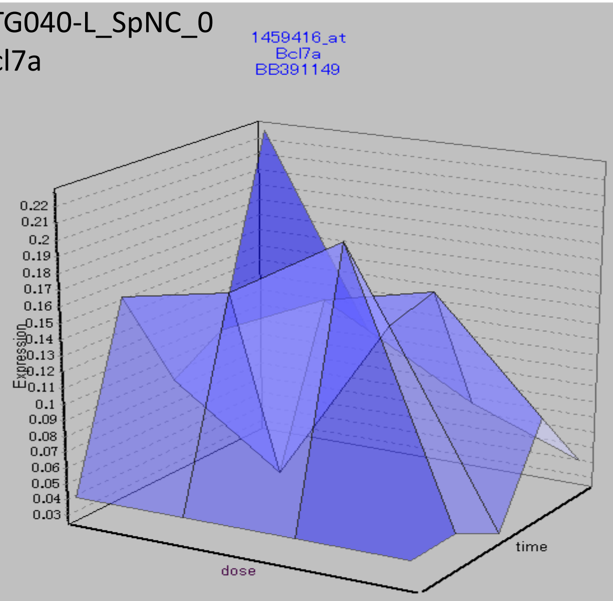

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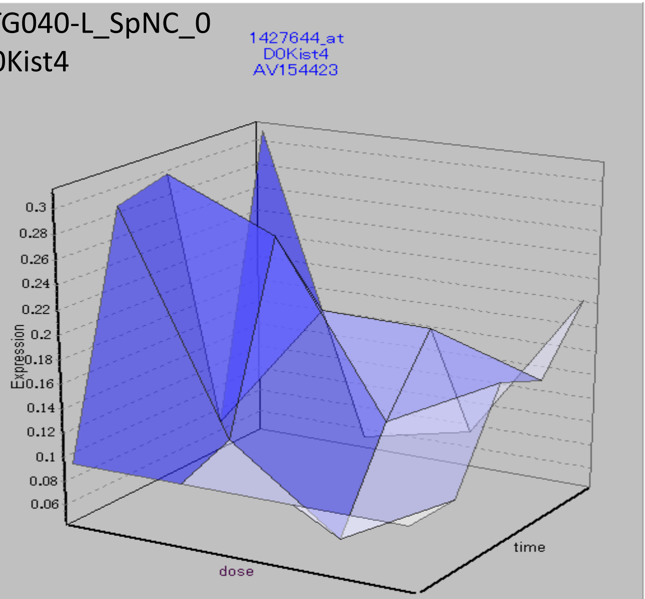

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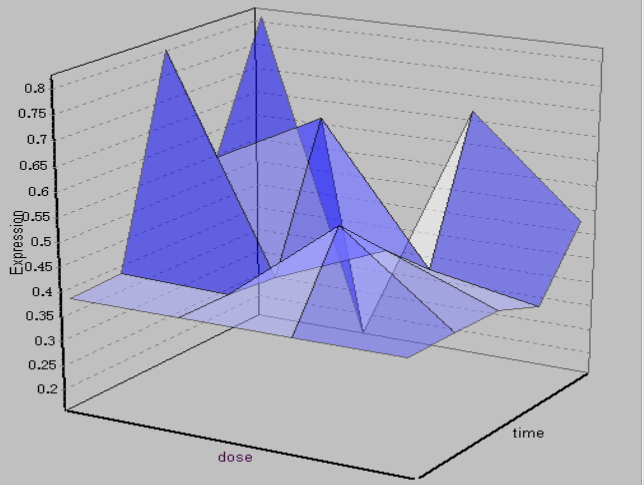

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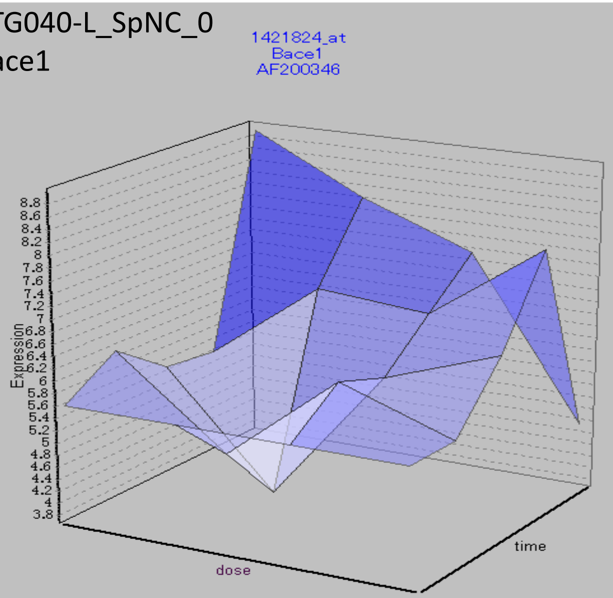

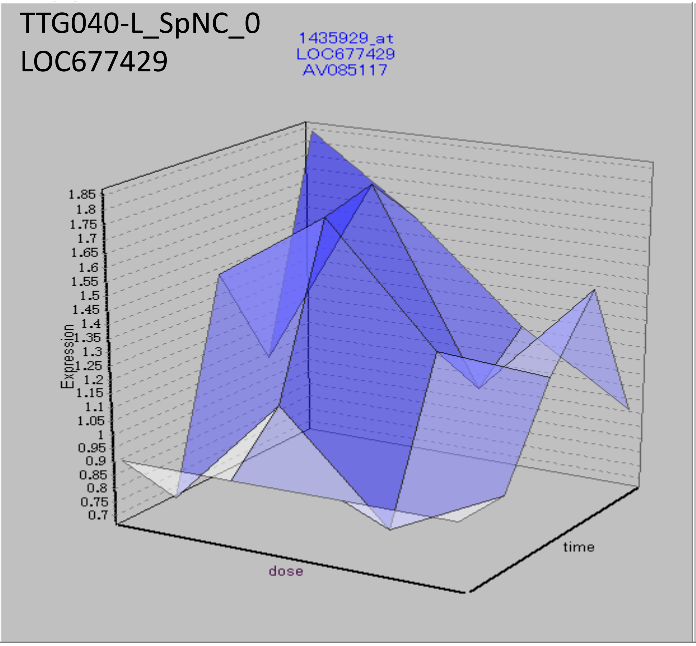
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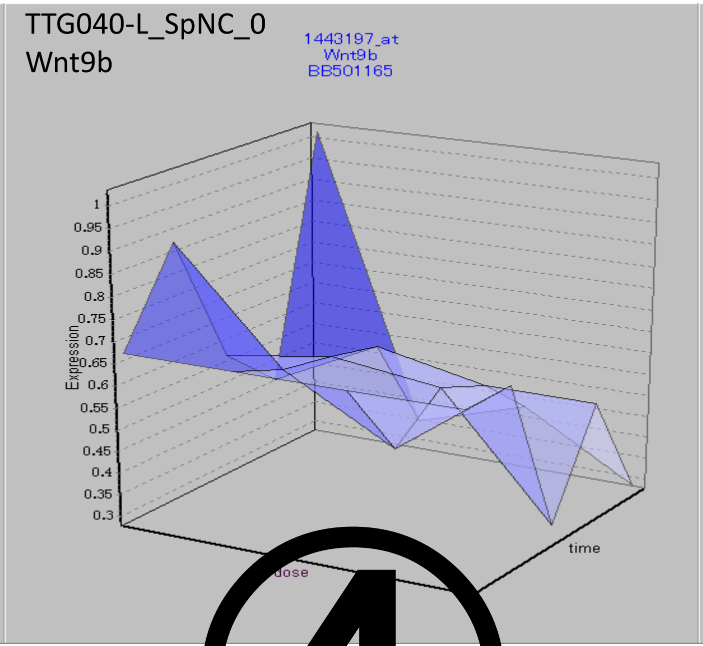
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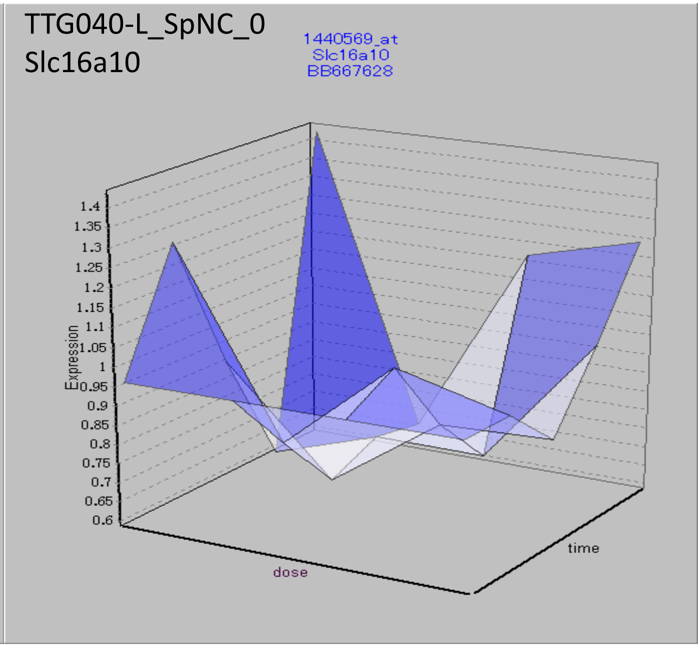
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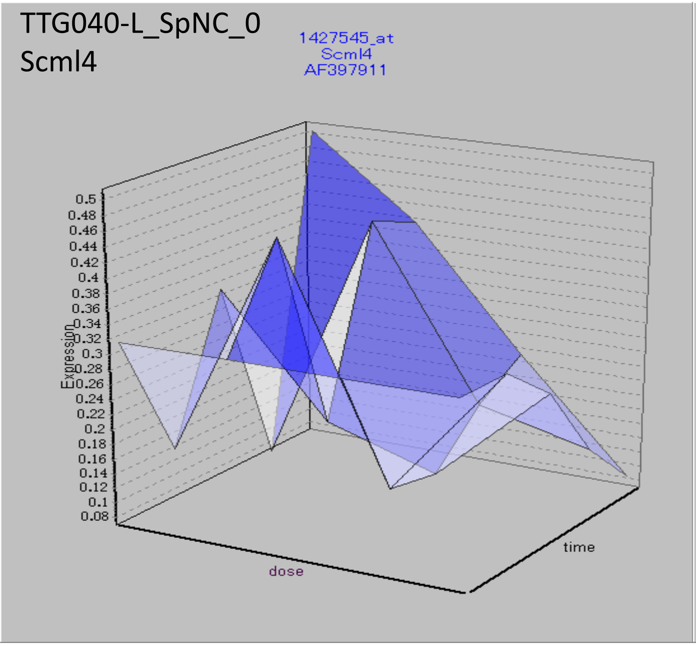
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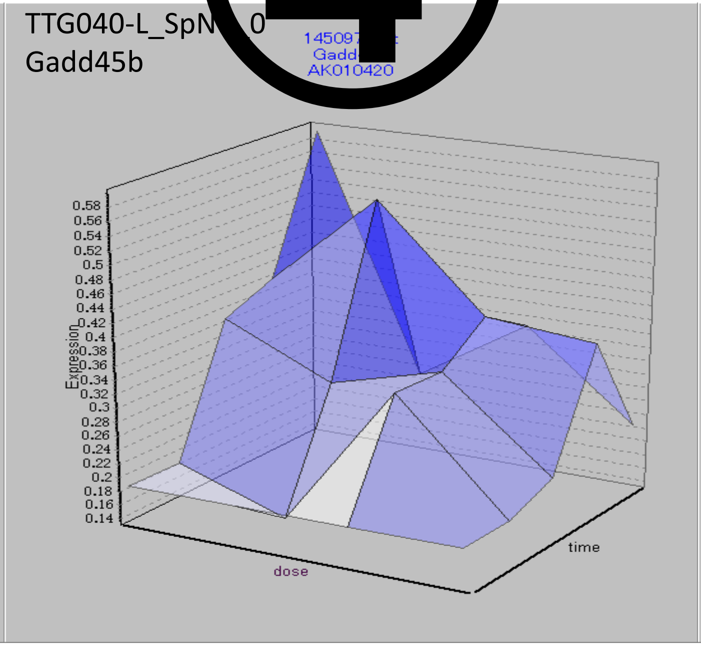
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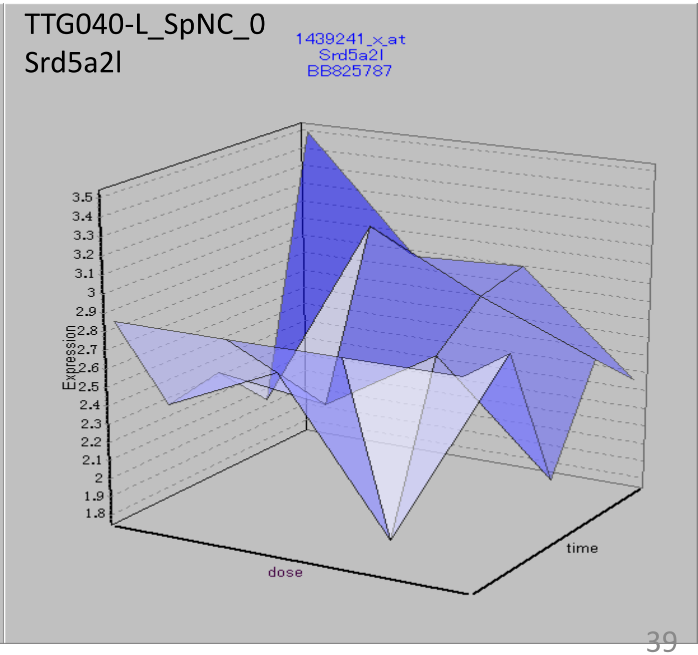
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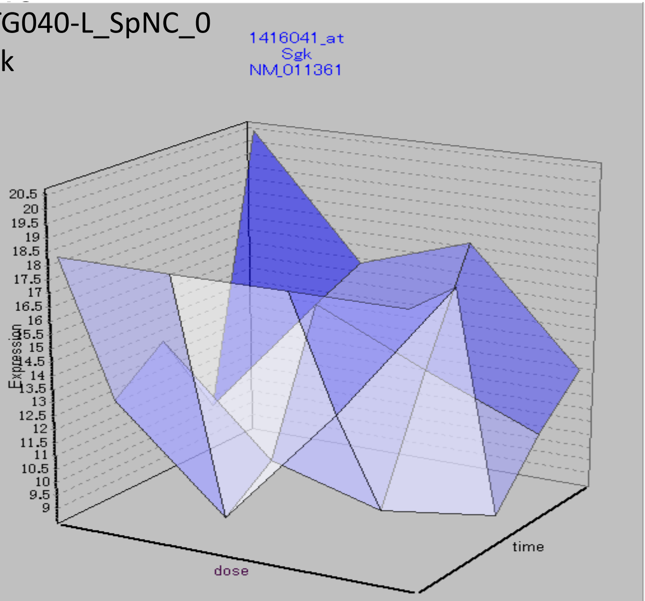
Sgk



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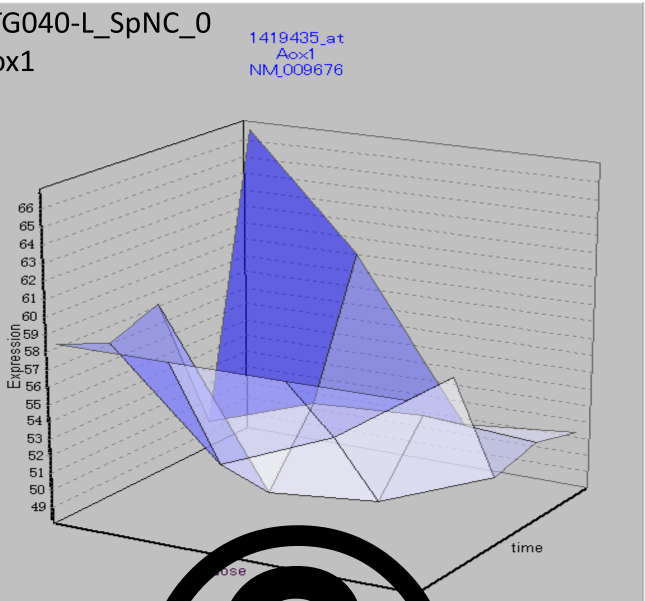

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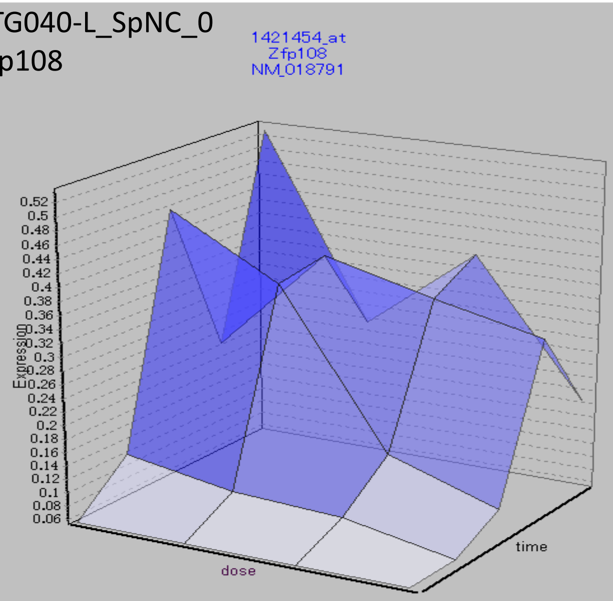

③

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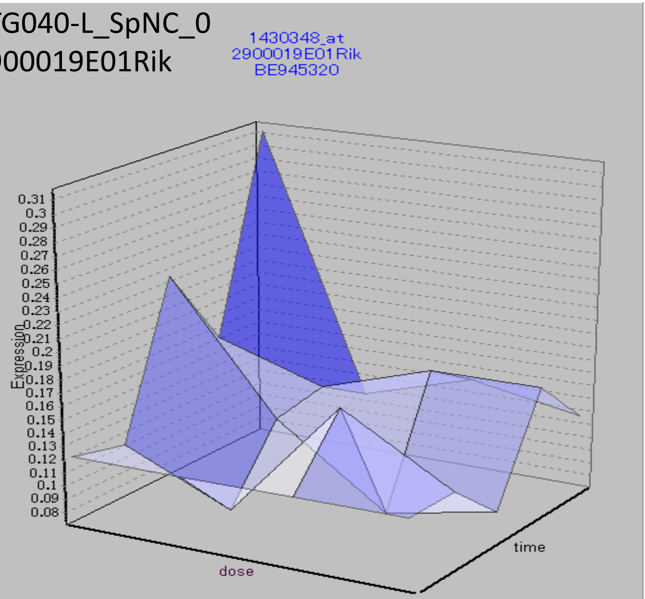

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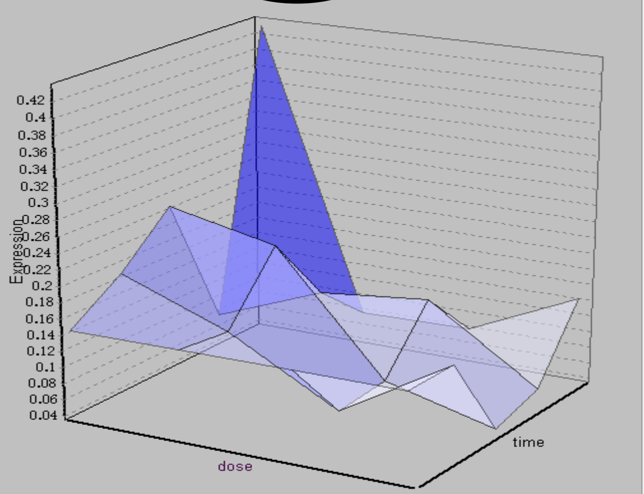

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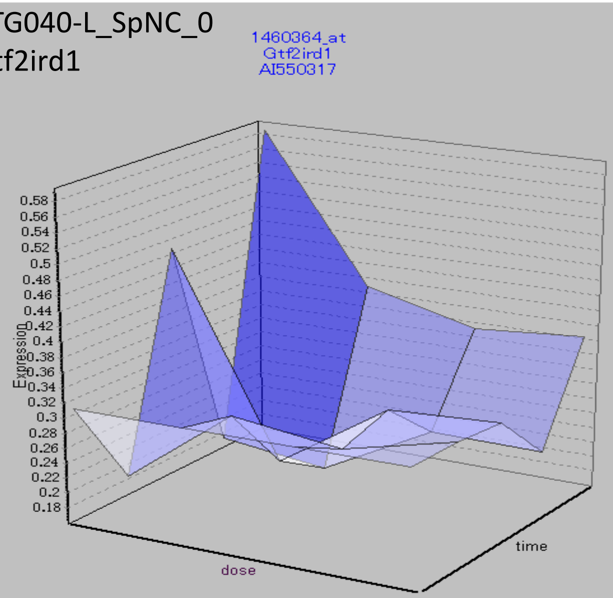

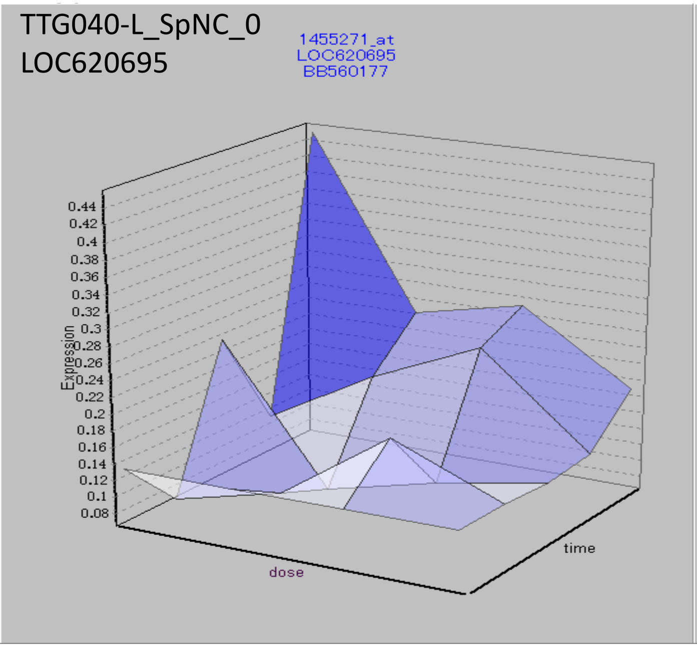
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LOC620695
1455271_at
LOC620695
BB560177
Expression
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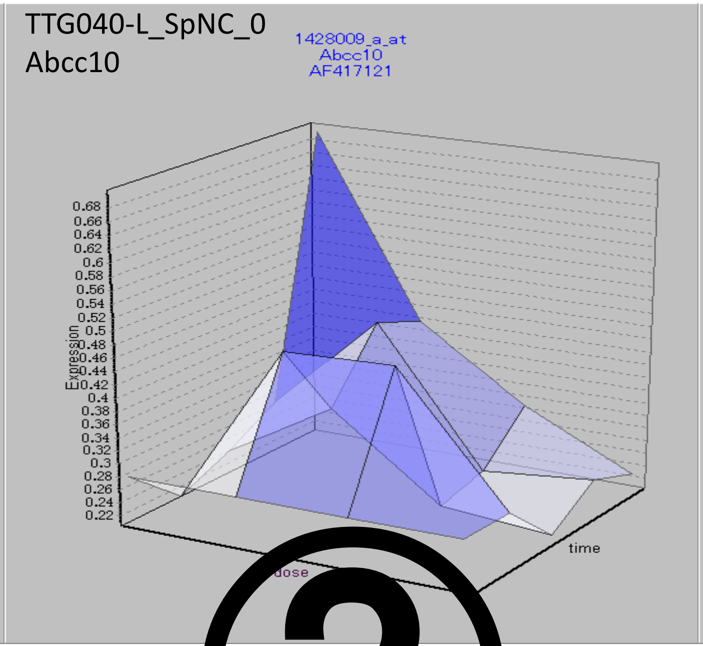
dose
time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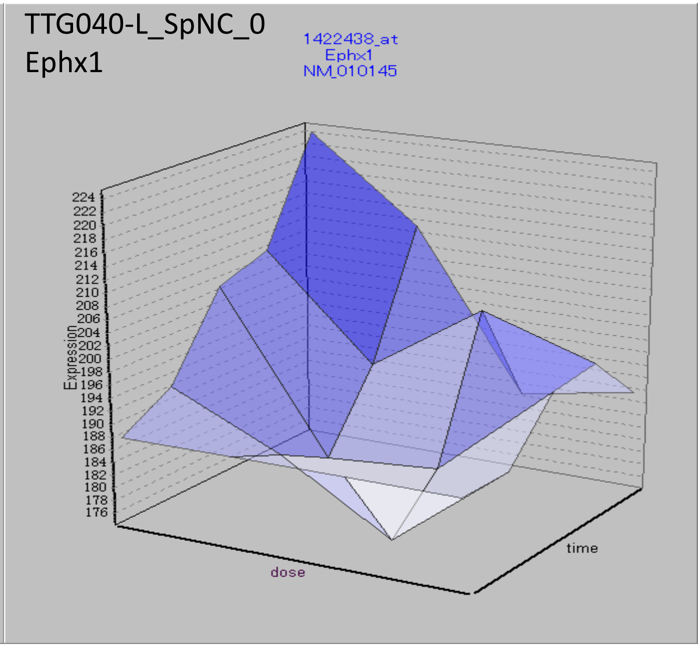
dose
time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Ephx1
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Expression
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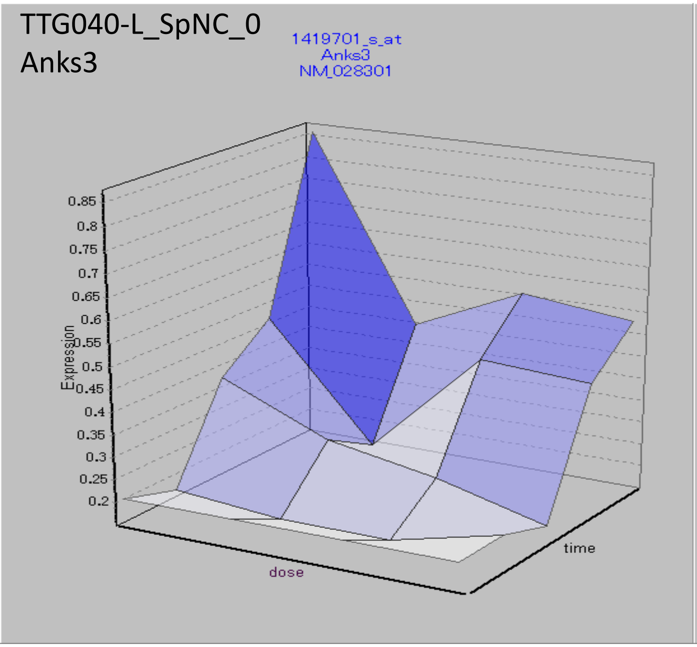
dose
time

②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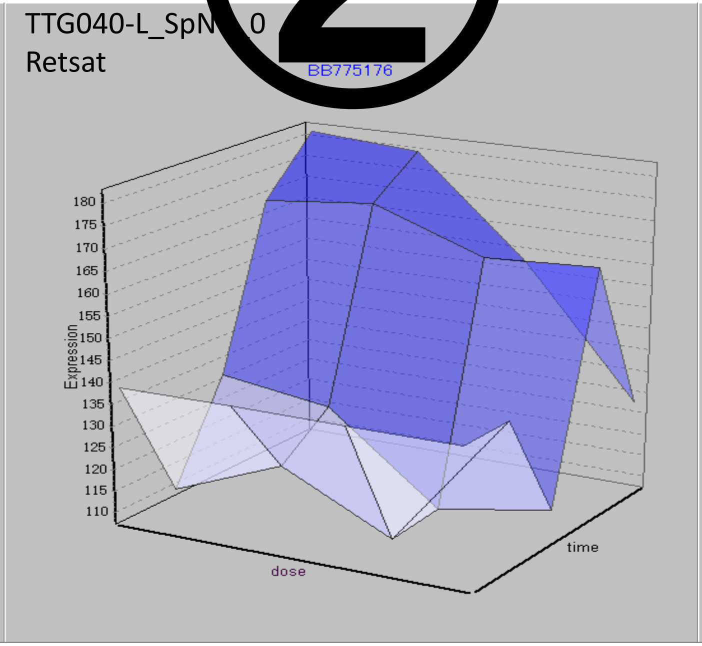
dose
time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Retsat
BB775176
Expression
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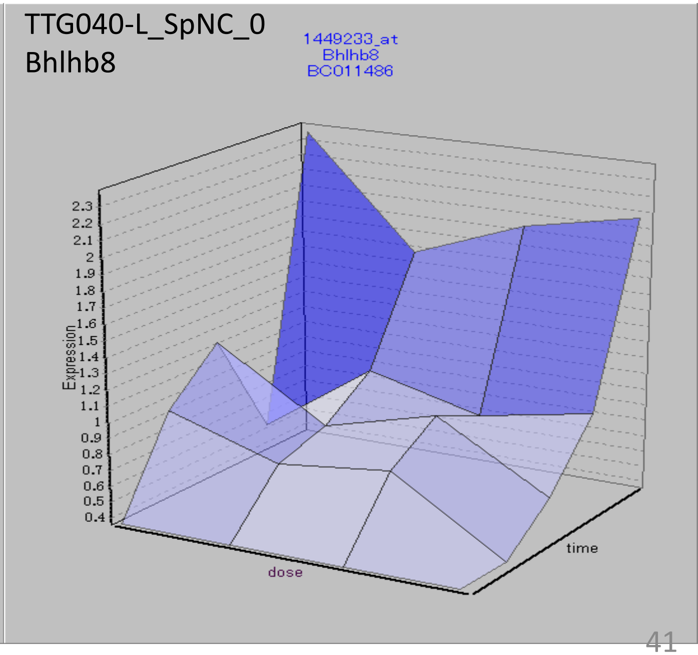
dose
time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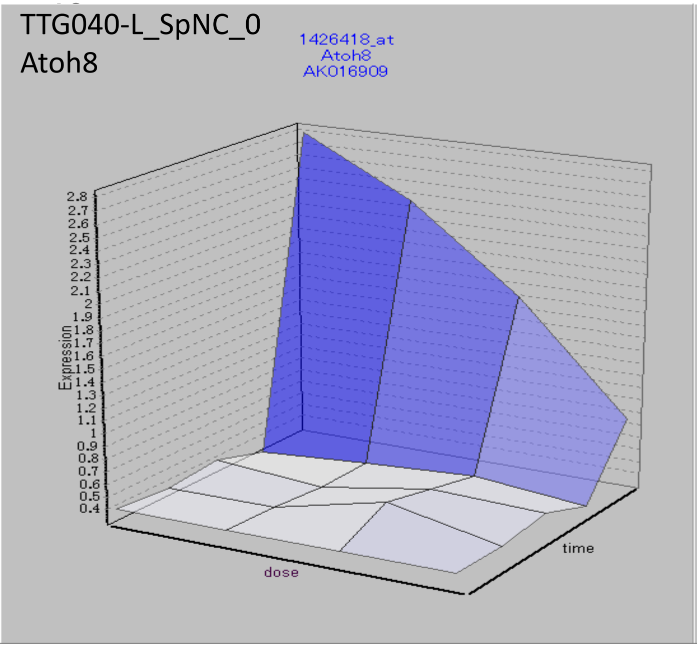
Bhlhb8
1449233_at
Bhlhb8
BC011486
Expression
2.3 2.2 2.1 2 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1 0.9 0.8 0.7 0.6 0.5 0.4
dose
time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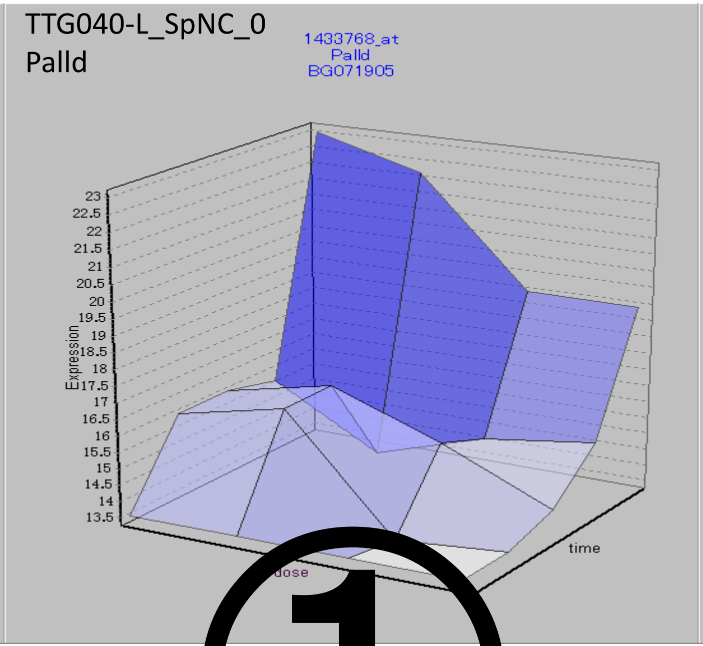
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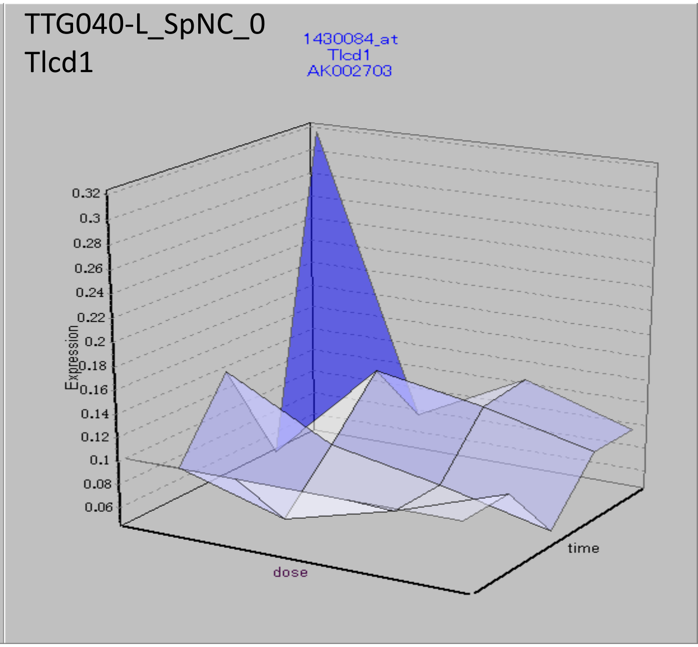
Palld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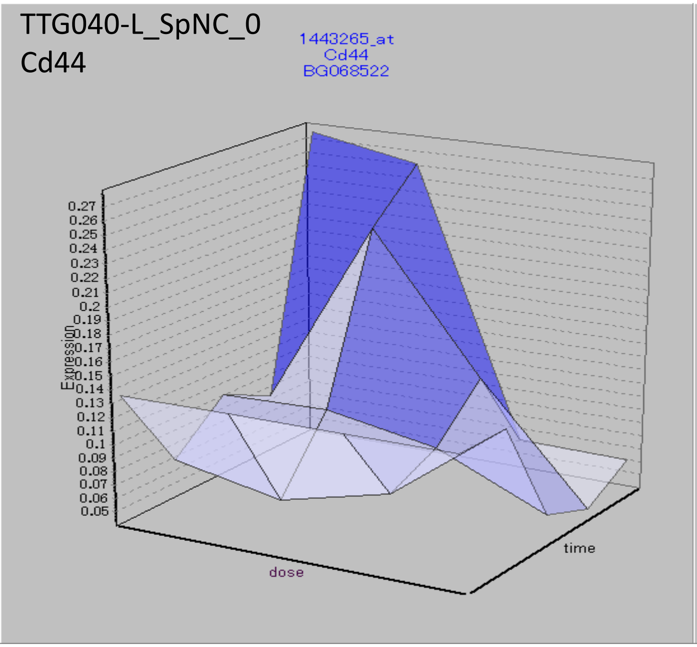
①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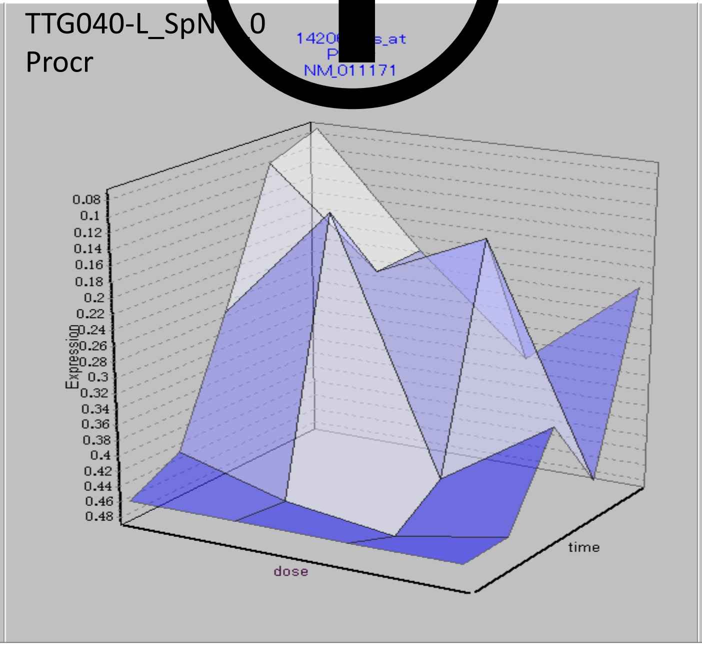
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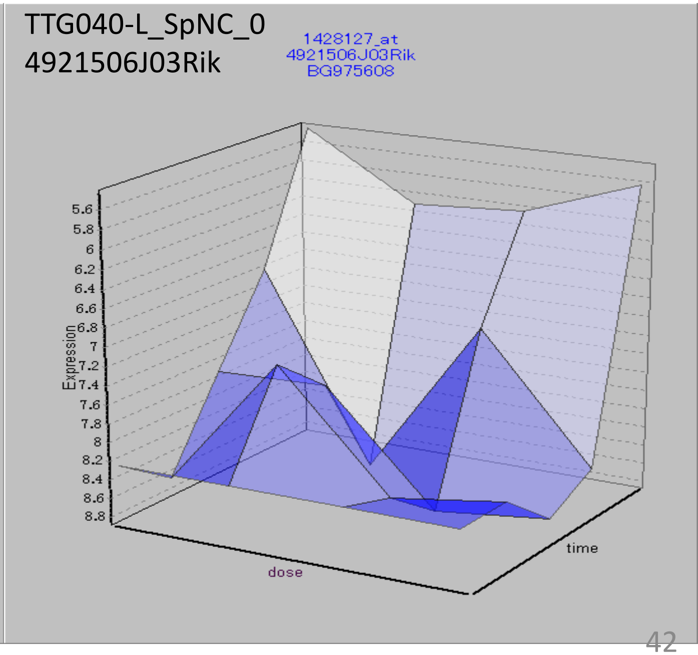
Procr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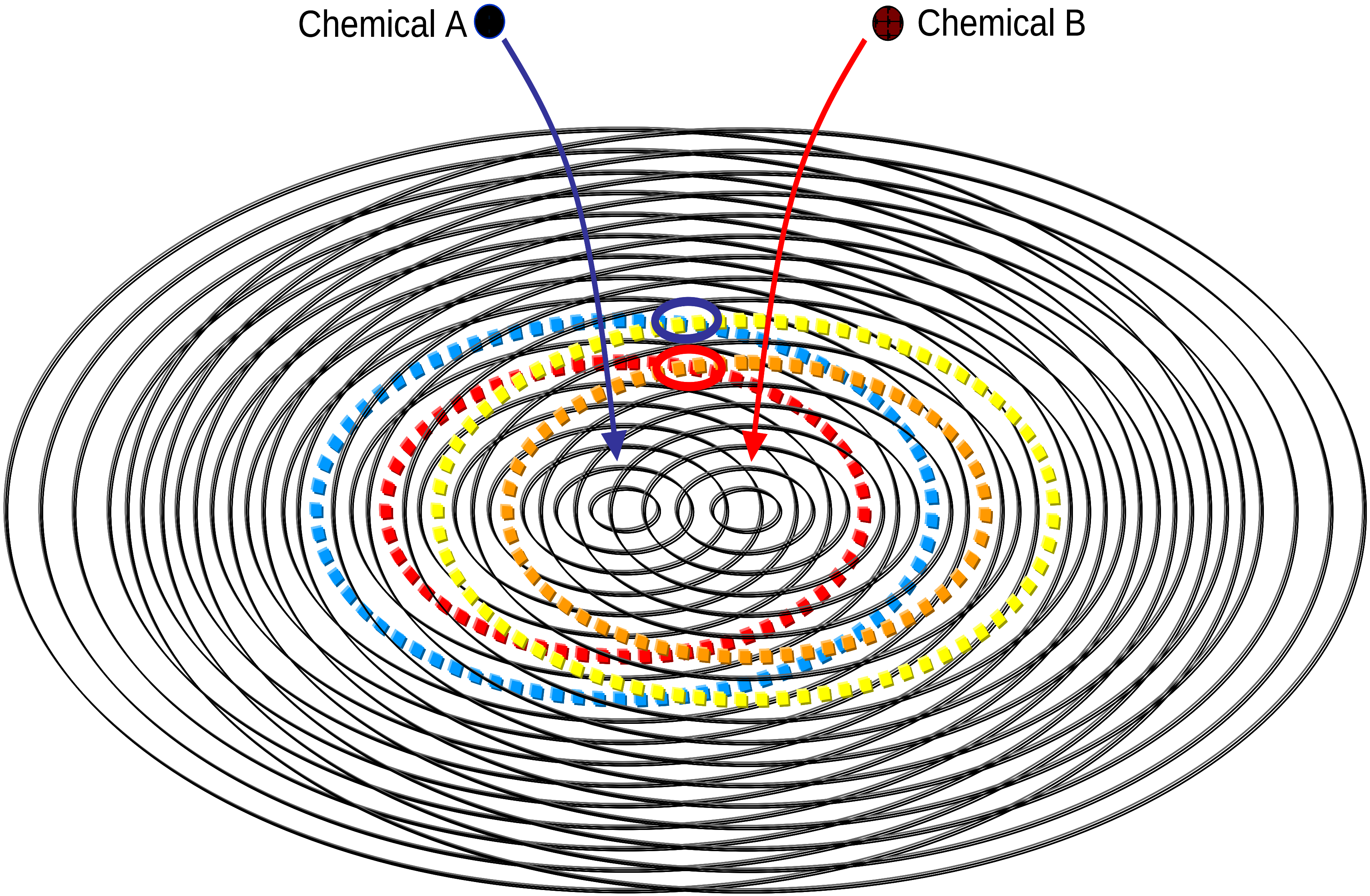
4921506J03Rik

- Data analysis

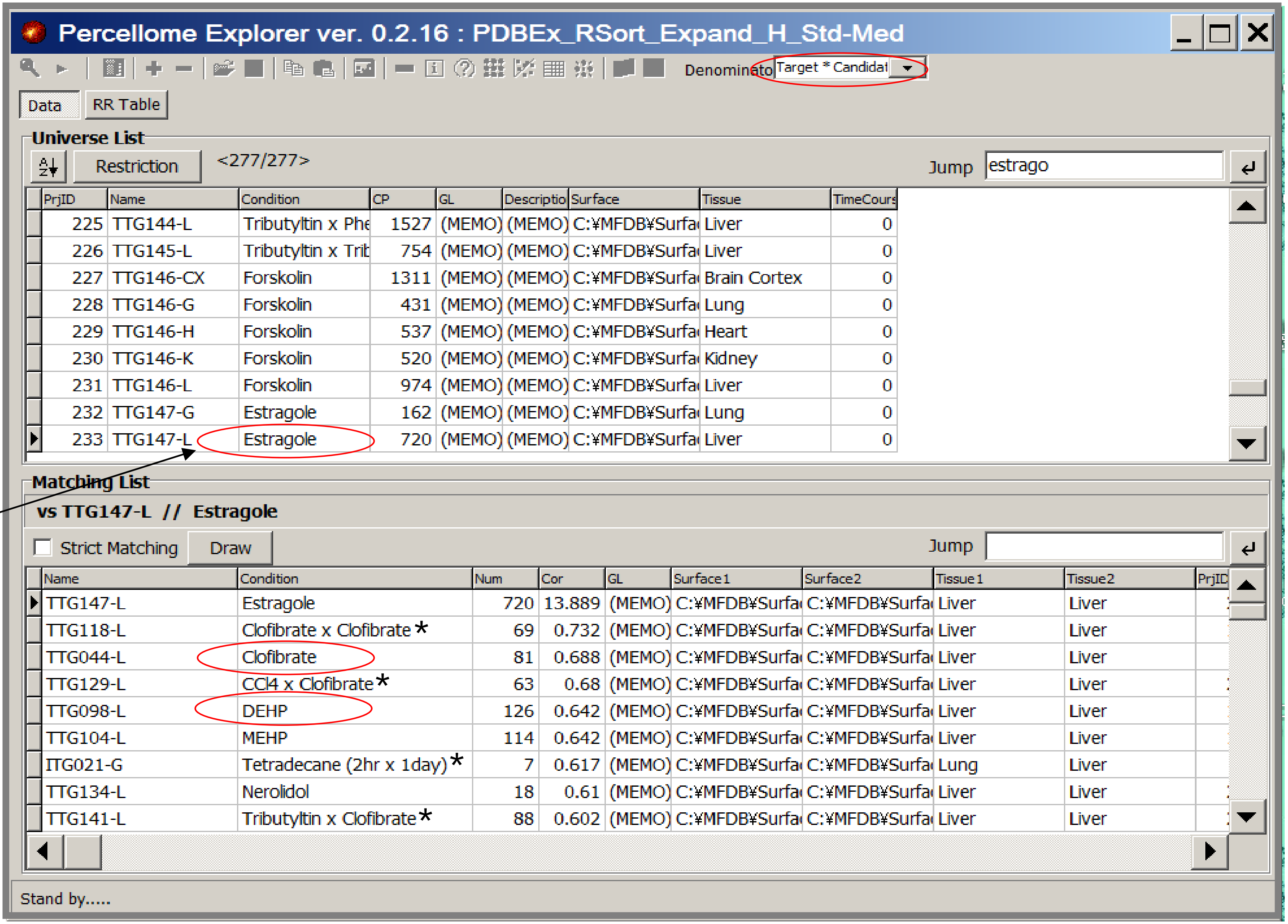

Chemical A
Chemical B

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


Percellome Explorer ver. 0.2.16 : PDBEx_RSort_Expand_H_Std-Med
Denominato
Target * Candidat
Data
RR Table
Universe List
Restriction
<277/277>
Jump
estrago
Matching List
vs TTG147-L // Estragole
☐ Strict Matching
Draw
Jump
Stand by.....

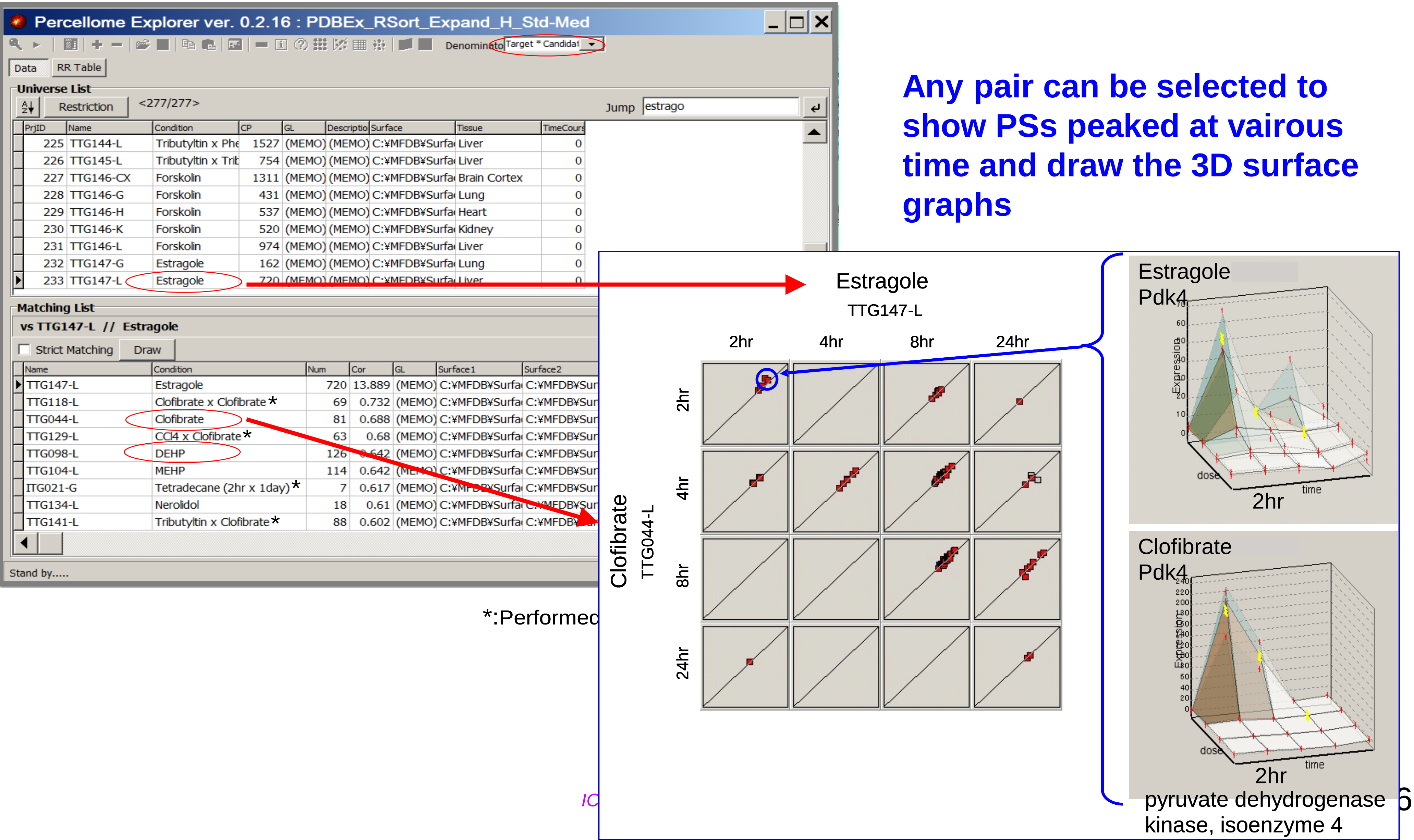
PrjID	Name	Condition	CP	GL	Descriptio	Surface	Tissue	TimeCours
225	TTG144-L	Tributyltin x Phe	1527	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Liver	0
226	TTG145-L	Tributyltin x Trib	754	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Liver	0
227	TTG146-CX	Forskolin	1311	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Brain Cortex	0
228	TTG146-G	Forskolin	431	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Lung	0
229	TTG146-H	Forskolin	537	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Heart	0
230	TTG146-K	Forskolin	520	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Kidney	0
231	TTG146-L	Forskolin	974	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Liver	0
232	TTG147-G	Estragole	162	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Lung	0
233	TTG147-L	Estragole	720	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Liver	0

Name	Condition	Num	Cor	GL	Surface1	Surface2	Tissue1	Tissue2	PrjID
TTG147-L	Estragole	720	13.889	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Surfa	Liver	Liver	
TTG118-L	Clofibrate x Clofibrate*	69	0.732	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Surfa	Liver	Liver	
TTG044-L	Clofibrate	81	0.688	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Surfa	Liver	Liver	
TTG129-L	CCl4 x Clofibrate*	63	0.68	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Surfa	Liver	Liver	
TTG098-L	DEHP	126	0.642	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Surfa	Liver	Liver	
TTG104-L	MEHP	114	0.642	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Surfa	Liver	Liver	
ITG021-G	Tetradecane (2hr x 1day)*	7	0.617	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Surfa	Lung	Liver	
TTG134-L	Nerolidol	18	0.61	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Surfa	Liver	Liver	
TTG141-L	Tributyltin x Clofibrate*	88	0.602	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Surfa	Liver	Liver	

*:Performed by different protocols

PercellomeExplorer Program by K Aisaki

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

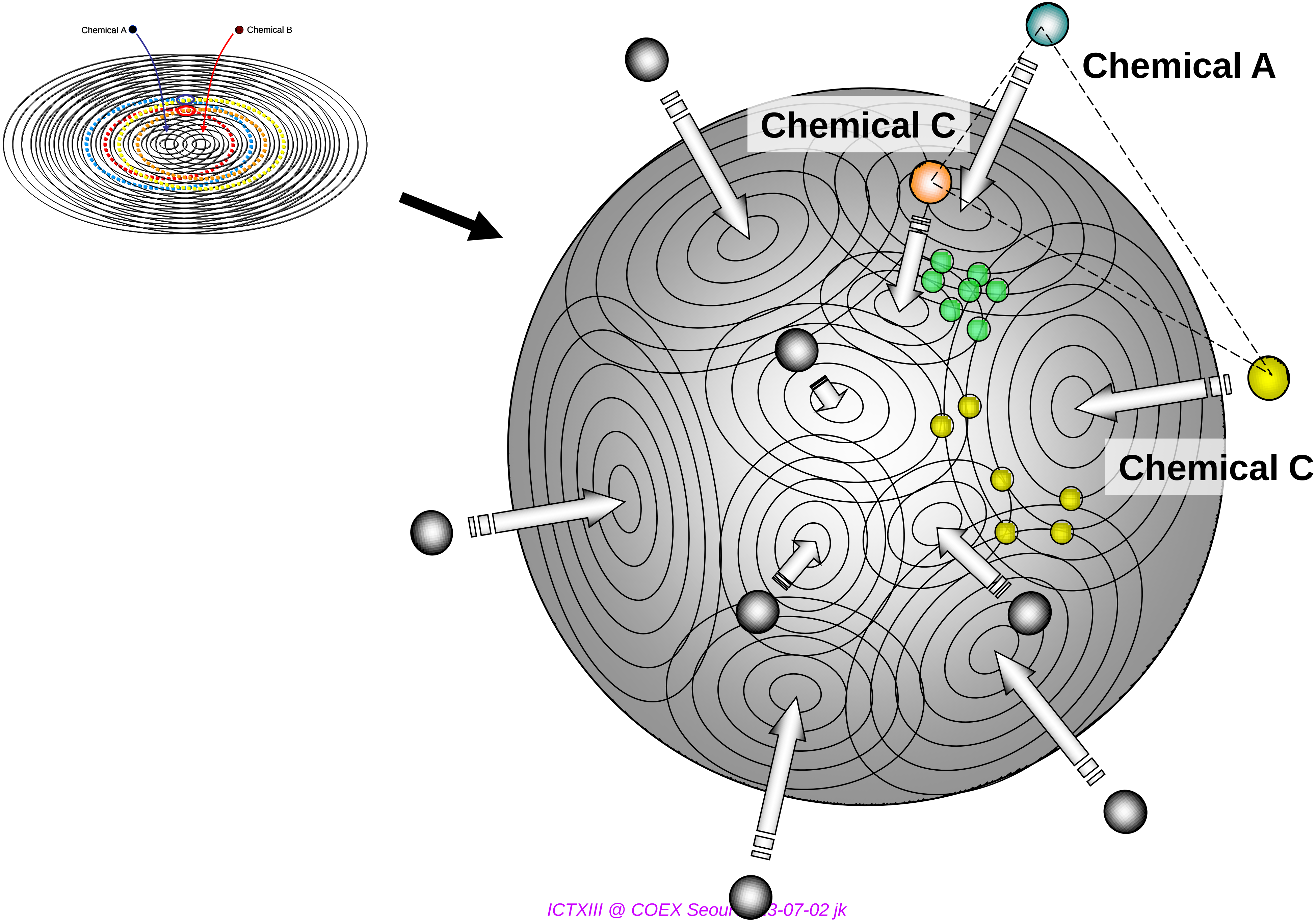

Percellome Explorer ver. 0.2.16 : PDBEx_RSort_Expand_H_Std-Med
Denominato
Target * Candidat
Data
RR Table
Universe List
Restriction
<277/277>
Jump
estrago
Matching List
vs TTG147-L // Estragole
☐ Strict Matching
Draw
Stand by.....
Estragole
TTG147-L
2hr
4hr
8hr
24hr
Clofibrate
TTG044-L
2hr
4hr
8hr
24hr
Estragole
Pdk4
Expression
dose
time
2hr
Clofibrate
Pdk4
Expression
dose
time
2hr

PrjID	Name	Condition	CP	GL	Descriptio	Surface	Tissue	TimeCours
225	TTG144-L	Tributyltin x Phe	1527	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Liver	0
226	TTG145-L	Tributyltin x Trib	754	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Liver	0
227	TTG146-CX	Forskolin	1311	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Brain Cortex	0
228	TTG146-G	Forskolin	431	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Lung	0
229	TTG146-H	Forskolin	537	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Heart	0
230	TTG146-K	Forskolin	520	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Kidney	0
231	TTG146-L	Forskolin	974	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Liver	0
232	TTG147-G	Estragole	162	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Lung	0
233	TTG147-L	Estragole	720	(MEMO)	(MEMO)	C:¥MFDB¥Surfa	Liver	0

Name	Condition	Num	Cor	GL	Surface1	Surface2
TTG147-L	Estragole	720	13.889	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Sur
TTG118-L	Clofibrate x Clofibrate *	69	0.732	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Sur
TTG044-L	Clofibrate	81	0.688	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Sur
TTG129-L	CCl4 x Clofibrate *	63	0.68	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Sur
TTG098-L	DEHP	126	0.642	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Sur
TTG104-L	MEHP	114	0.642	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Sur
ITG021-G	Tetradecane (2hr x 1day) *	7	0.617	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Sur
TTG134-L	Nerolidol	18	0.61	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Sur
TTG141-L	Tributyltin x Clofibrate *	88	0.602	(MEMO)	C:¥MFDB¥Surfa	C:¥MFDB¥Sur

*:Performed

pyruvate dehydrogenase kinase, isoenzyme 4

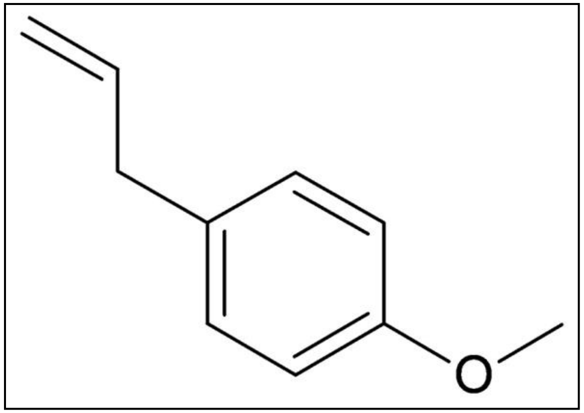

Chemical A
Chemical B
Chemical A
Chemical C
Chemical C

- Percellome data analysis strategy
  - Comprehensive
  - Unsupervised

- Comprehensive Network Drawing

July 3 (Wed) 15:30～17:00
OP2-4 Satoshi Kitajima

Flavor "estragole"



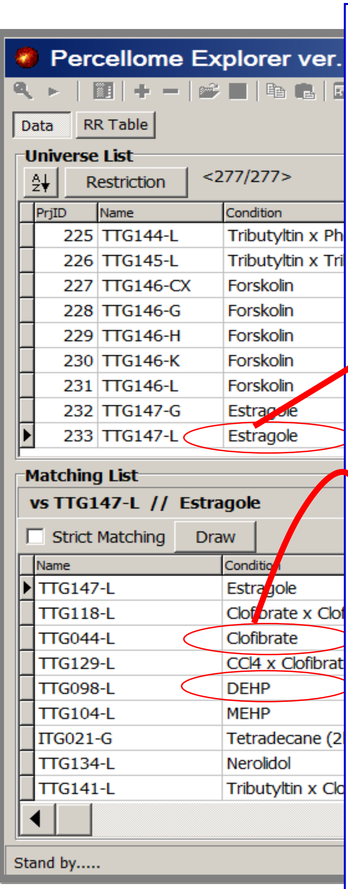
(1-allyl-4-methoxybenzene)
(CAS No.: 140-67-0)

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



Universe List

<277/277>

PrjID	Name	Condition
225	TTG144-L	Tributyltin x Ph
226	TTG145-L	Tributyltin x Tri
227	TTG146-CX	Forskolin
228	TTG146-G	Forskolin
229	TTG146-H	Forskolin
230	TTG146-K	Forskolin
231	TTG146-L	Forskolin
232	TTG147-G	Estragole
233	TTG147-L	Estragole

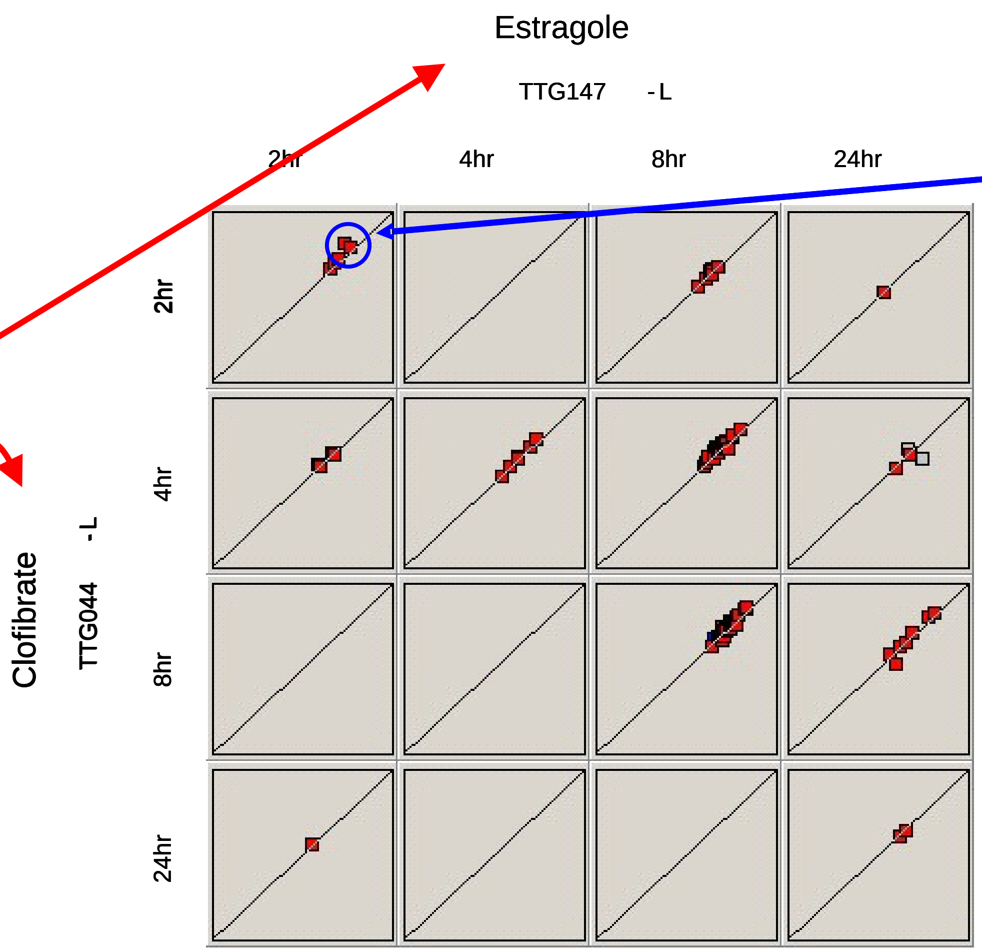
Matching List

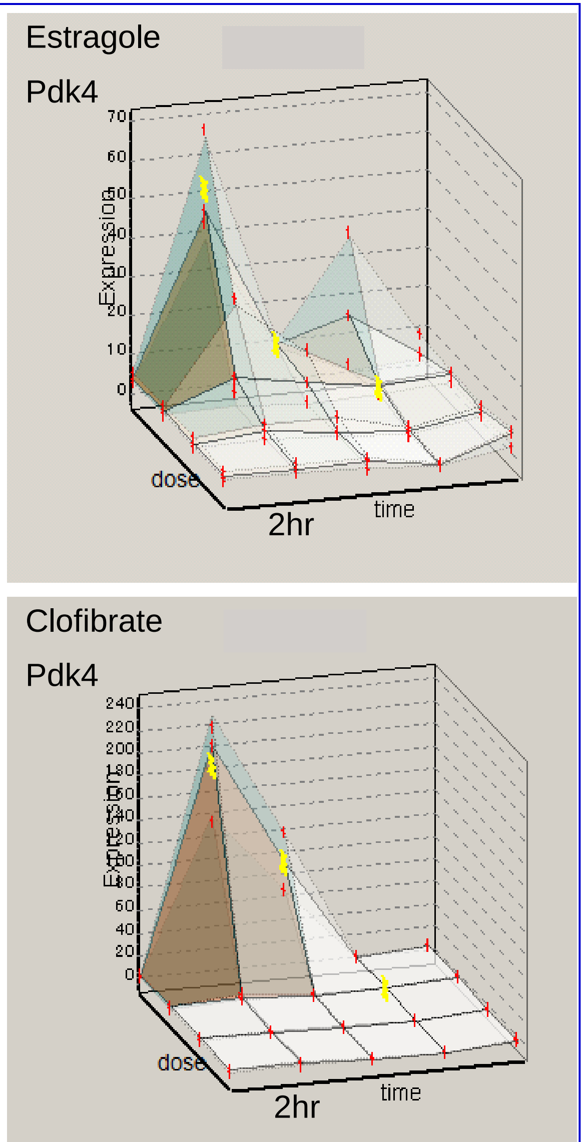
vs TTG147-L // Estragole

☐ Strict Matching

Name	Condition
TTG147-L	Estragole
TTG118-L	Clofibrate x Clof
TTG044-L	Clofibrate
TTG129-L	CCl4 x Clofibrat
TTG098-L	DEHP
TTG104-L	MEHP
ITG021-G	Tetradecane (2
TTG134-L	Nerolidol
TTG141-L	Tributyltin x Clo

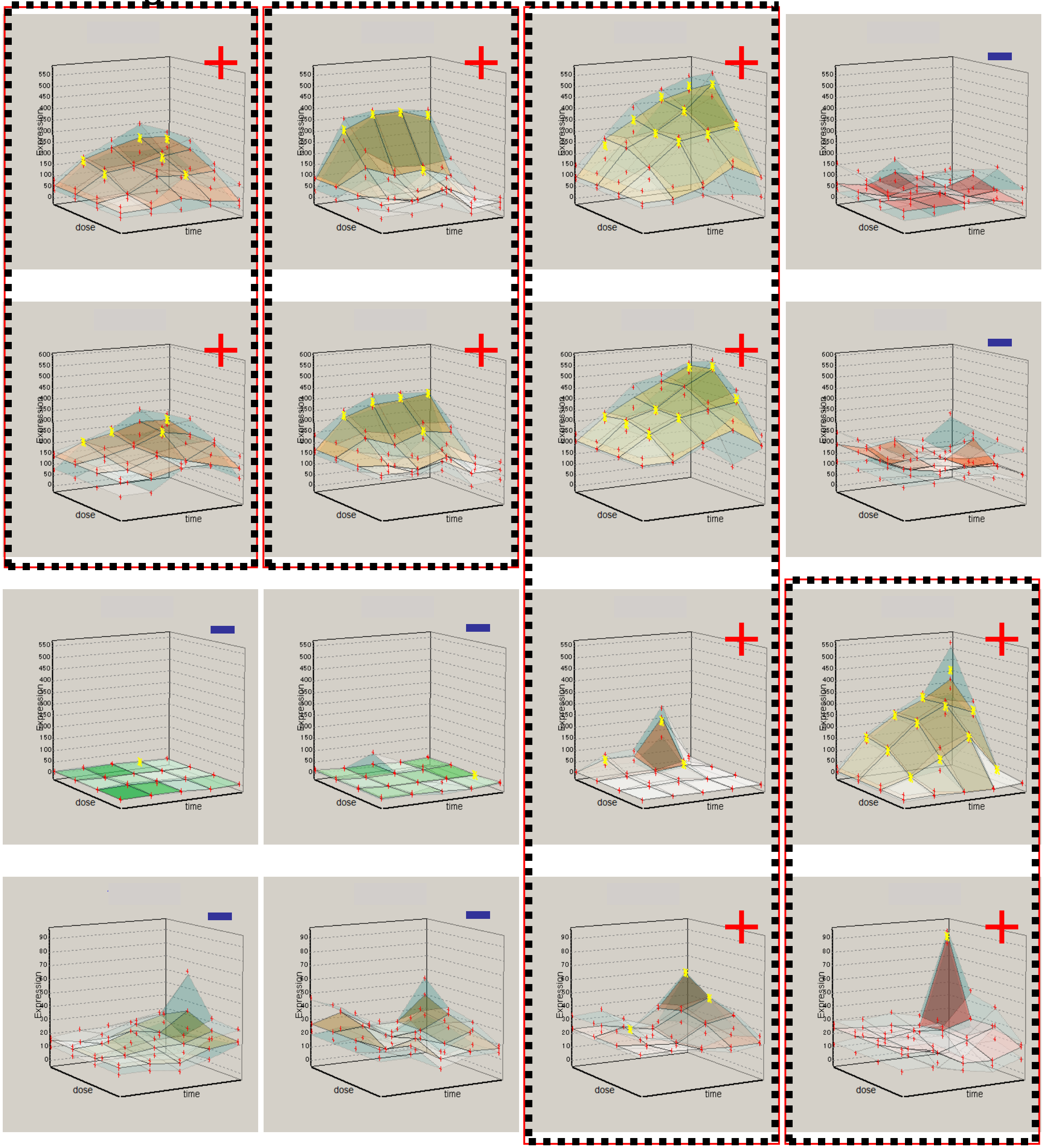
Stand by.....





pyruvate dehydrogenase kinase, isoenzyme 4

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

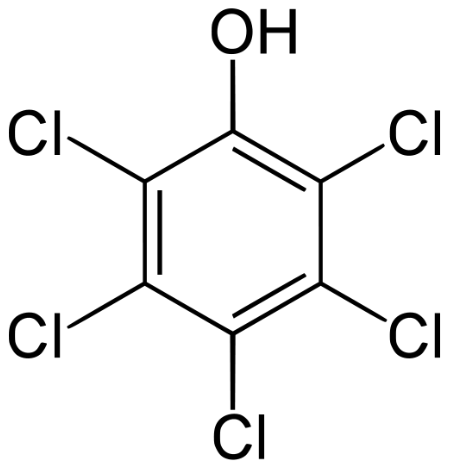
Cyp2b10
cytochrome P450,
family 2,
subfamily b,
polypeptide 10

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.

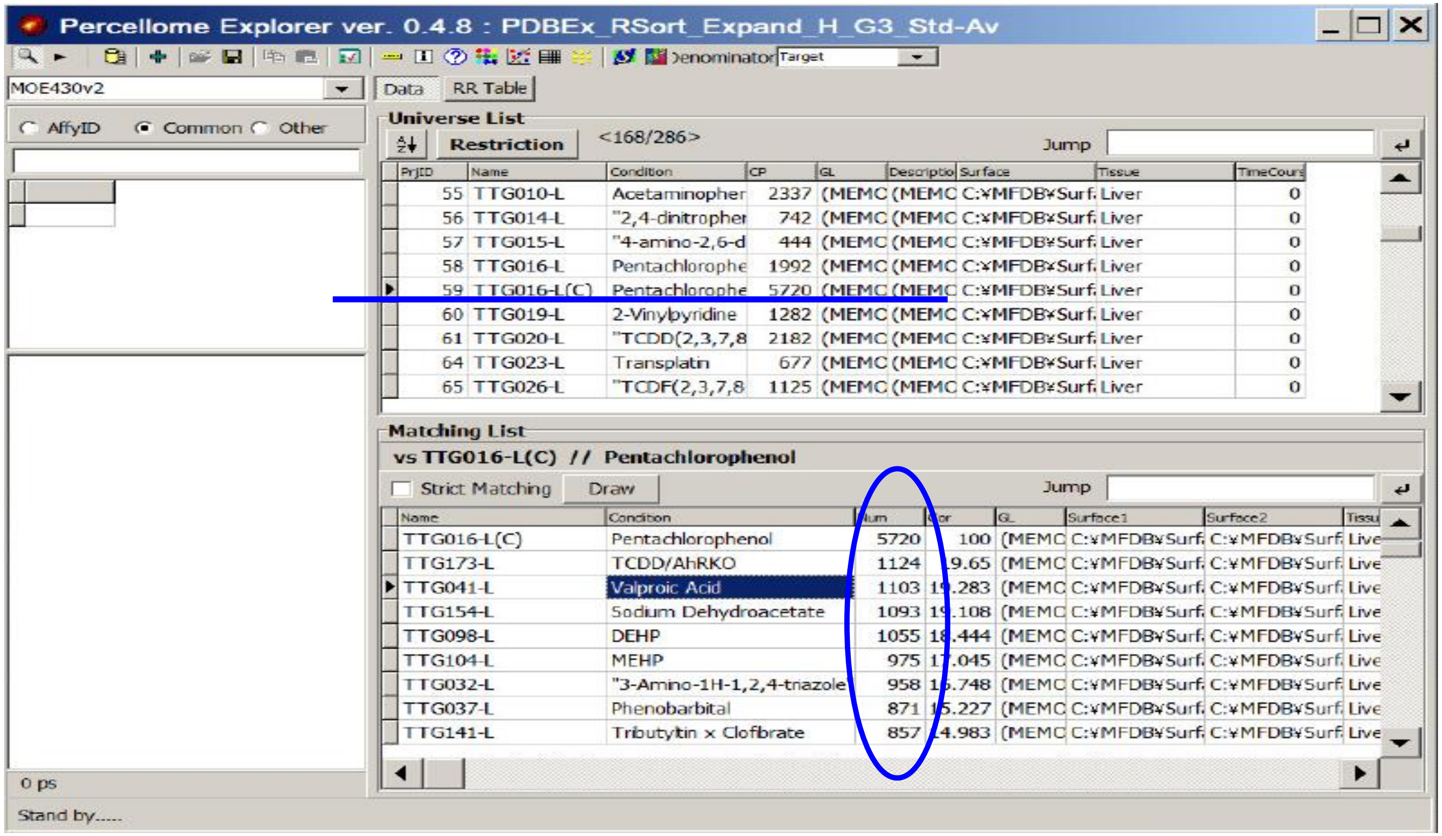
Chronic symptoms:

Liver, kidney, neural

Liver tumor induction

J Tox Sci, Vol. 38-4, 2013, in press

Figure 2


Percellome Explorer ver. 0.4.8 : PDBEx_RSort_Expand_H_G3_Std-Av
Denominator
MOE430v2
○ AffyID ● Common ○ Other
Data
RR Table
Restriction
Jump
Draw
0 ps
Stand by.....

Denominator: Target

Universe List

<168/286>

PrjID	Name	Condition	CP	GL	Descriptio	Surface	Tissue	TimeCours
55	TTG010-L	Acetaminopher	2337	(MEMC	(MEMC	C:¥MFDB¥Surf.	Liver	0
56	TTG014-L	"2,4-dinitropher	742	(MEMC	(MEMC	C:¥MFDB¥Surf.	Liver	0
57	TTG015-L	"4-amino-2,6-d	444	(MEMC	(MEMC	C:¥MFDB¥Surf.	Liver	0
58	TTG016-L	Pentachlorophe	1992	(MEMC	(MEMC	C:¥MFDB¥Surf.	Liver	0
59	TTG016-L(C)	Pentachlorophe	5720	(MEMC	(MEMC	C:¥MFDB¥Surf.	Liver	0
60	TTG019-L	2-Vinylpyridine	1282	(MEMC	(MEMC	C:¥MFDB¥Surf.	Liver	0
61	TTG020-L	"TCDD(2,3,7,8	2182	(MEMC	(MEMC	C:¥MFDB¥Surf.	Liver	0
64	TTG023-L	Transplatin	677	(MEMC	(MEMC	C:¥MFDB¥Surf.	Liver	0
65	TTG026-L	"TCDF(2,3,7,8	1125	(MEMC	(MEMC	C:¥MFDB¥Surf.	Liver	0

Matching List

vs TTG016-L(C) // Pentachlorophenol

☐ Strict Matching

Name	Condition	[illegible]um	[illegible]or	GL	Surface1	Surface2	Tissu
TTG016-L(C)	Pentachlorophenol	5720	100	(MEMC	C:¥MFDB¥Surf.	C:¥MFDB¥Surf.	Live
TTG173-L	TCDD/AhRKO	1124	[illegible]9.65	(MEMC	C:¥MFDB¥Surf.	C:¥MFDB¥Surf.	Live
TTG041-L	Valproic Acid	1103	1[illegible].283	(MEMC	C:¥MFDB¥Surf.	C:¥MFDB¥Surf.	Live
TTG154-L	Sodium Dehydroacetate	1093	1[illegible].108	(MEMC	C:¥MFDB¥Surf.	C:¥MFDB¥Surf.	Live
TTG098-L	DEHP	1055	1[illegible].444	(MEMC	C:¥MFDB¥Surf.	C:¥MFDB¥Surf.	Live
TTG104-L	MEHP	975	1[illegible].045	(MEMC	C:¥MFDB¥Surf.	C:¥MFDB¥Surf.	Live
TTG032-L	"3-Amino-1H-1,2,4-triazole"	958	1[illegible].748	(MEMC	C:¥MFDB¥Surf.	C:¥MFDB¥Surf.	Live
TTG037-L	Phenobarbital	871	1[illegible].227	(MEMC	C:¥MFDB¥Surf.	C:¥MFDB¥Surf.	Live
TTG141-L	Tributyltin x Clofibrate	857	[illegible]4.983	(MEMC	C:¥MFDB¥Surf.	C:¥MFDB¥Surf.	Live

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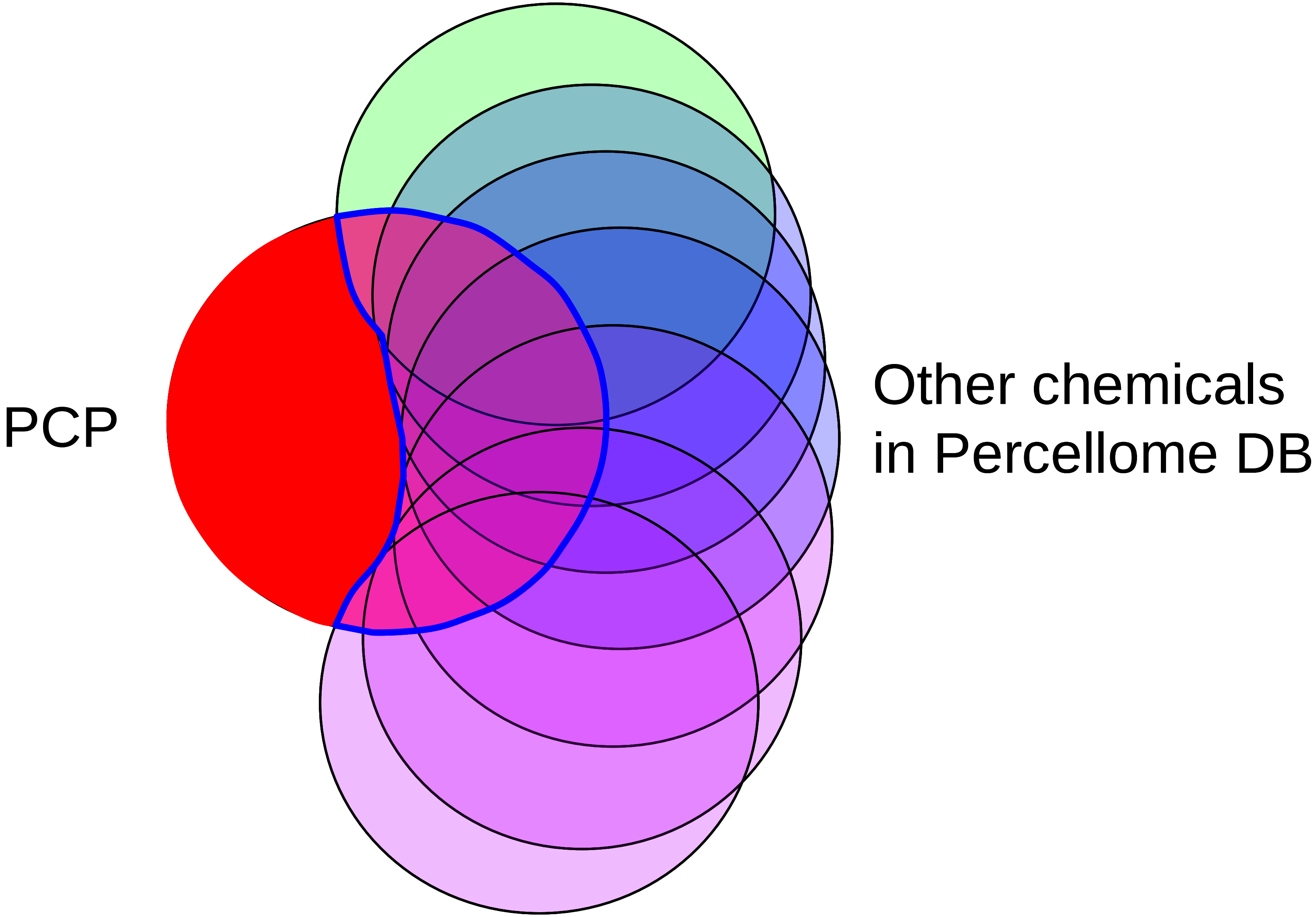
PCP
Other chemicals in Percellome DB

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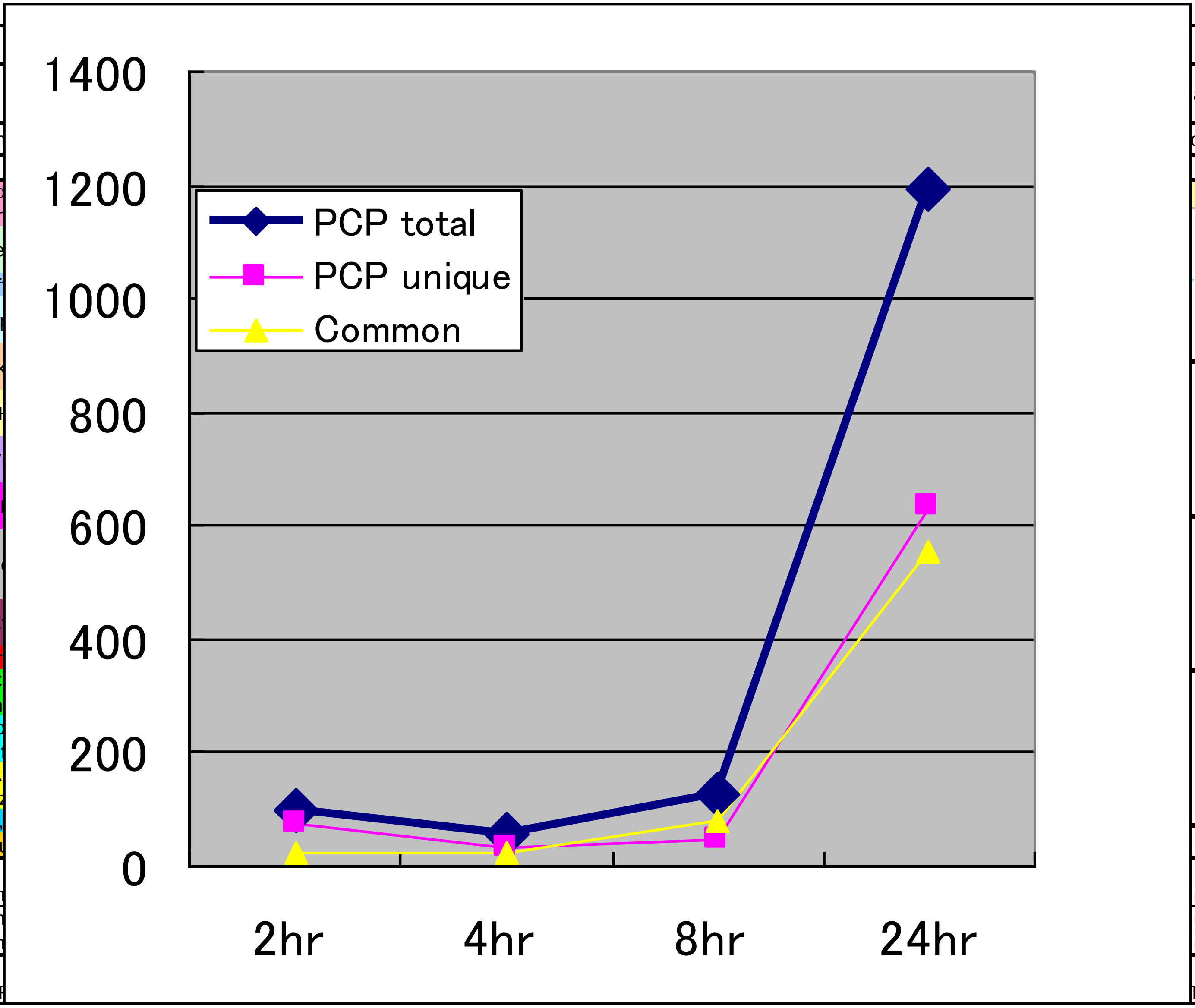
2hr			4hr			8hr			24hr		
Percellome No.	Treatment	PS	Percellome No.	Treatment	PS	Percellome No.	Treatment	PS	Percellome No.	Treatment	PS
TTG016-L(C)	Pentachlorophenol	98	TTG016-L(C)	Pentachlorophenol	55	TTG016-L(C)	Pentachlorophenol	127	TTG016-L(C)	Pentachlorophenol	1192
TTG154-L	Sodium Dehydroacetate	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

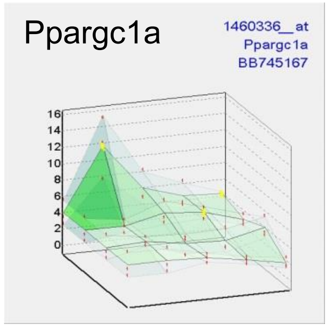
J Tox Sci, Vol. 38-4, 2013, in press

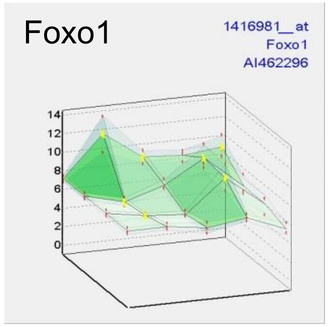


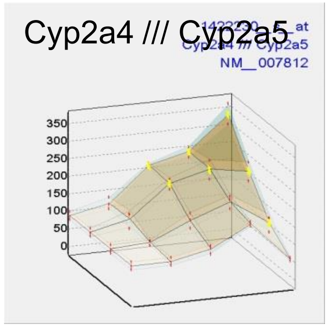
Percellome No.			PS
TTG016-L(C)	Per	orophenol	1192
TTG154-L	Soc Deh		258
TTG109-L	Ace	1,2,4-	212
TTG059-L	Caf		177
TTG041-L	Val	l	160
TTG062-L(C)	Dex		109
TTG098-L	DEI	sodium	103
TTG019-L	2-V	ne	94
TTG104-L	ME	ate	77
TTG165-L	Chl	d oil matter	71
TTG034-L	4-E		69
TTG166-L	Car	ne	47
TTG031-L	2-C dim		17
TTG141-L	Trib Clo		13
TTG032-L	3-A triaz		
TTG027-L	1,2,		
TTG160-L	5-flu		
	Sur	(Total)	636
	Sur	(Up)	636
	Sur	(Dn)	0
	PCF	T Sum	556

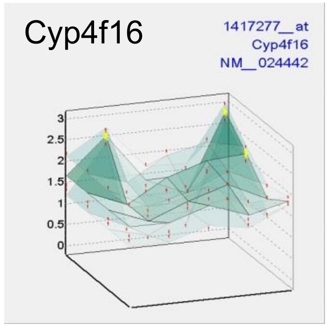
J Tox Sci, Vol. 38-4, 2013, in press

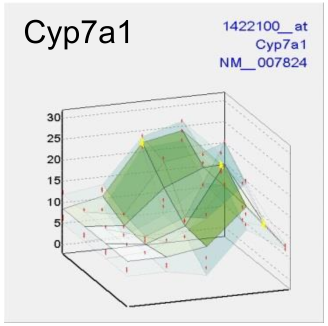
Genes Induced at 2, 4 or 8 hr

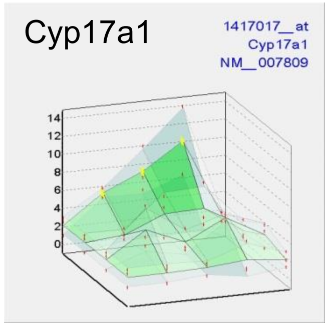


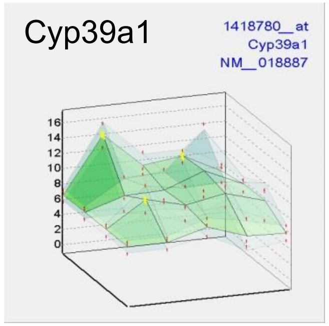


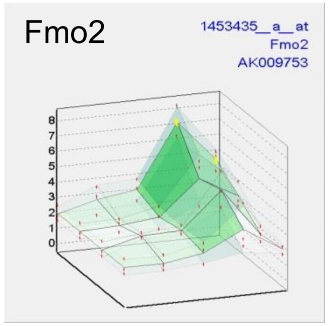


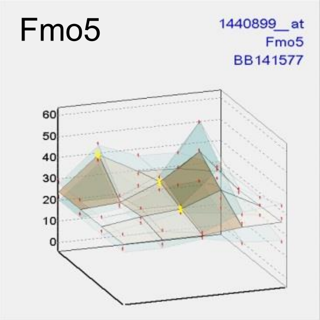


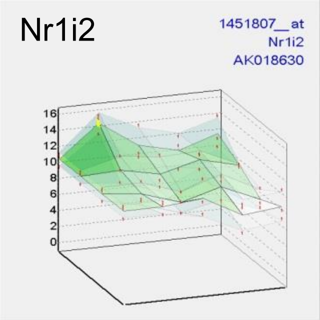


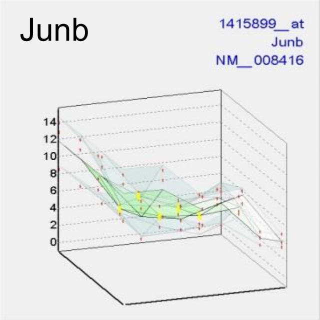


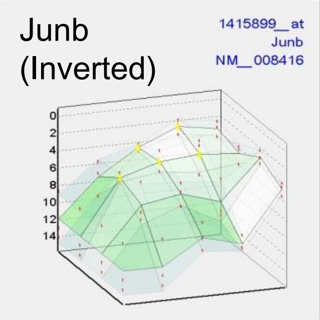


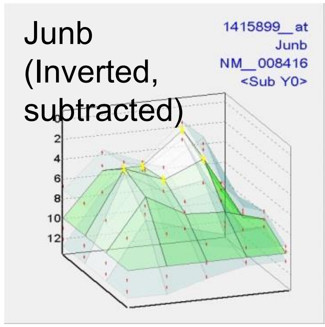


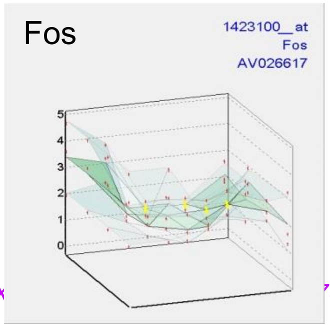


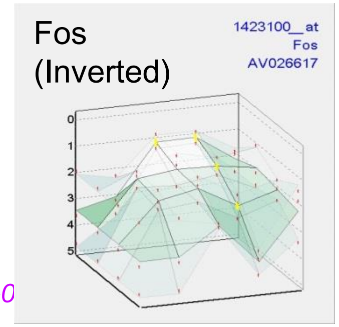


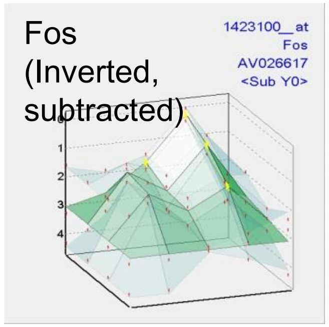












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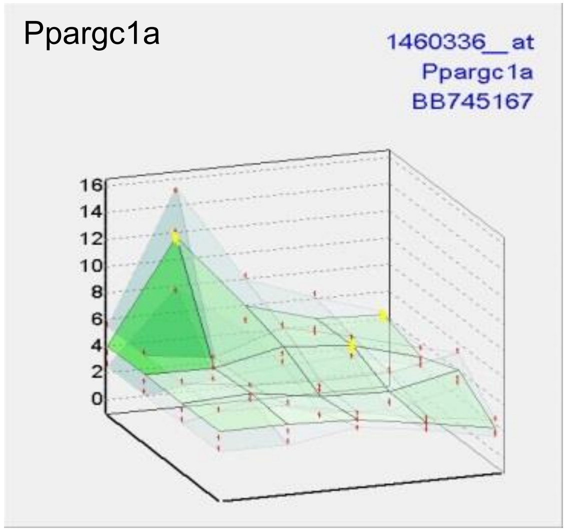
2, 4 or 8 hr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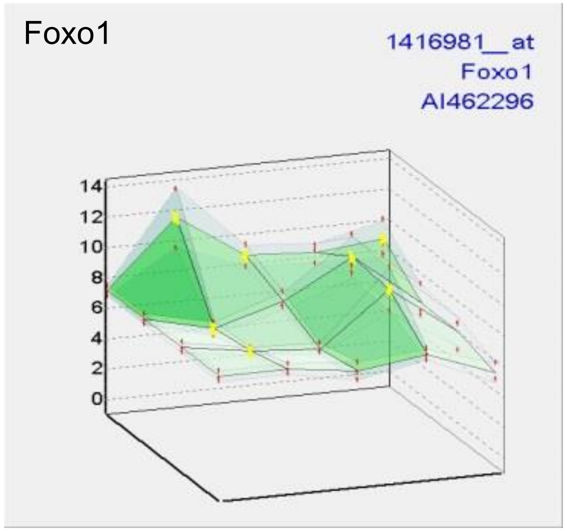
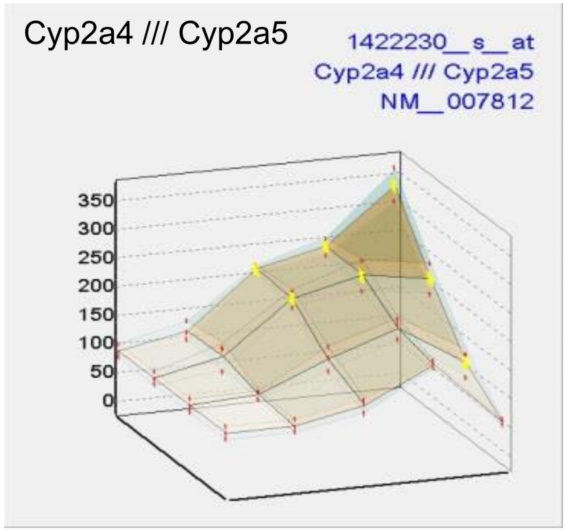

Foxo1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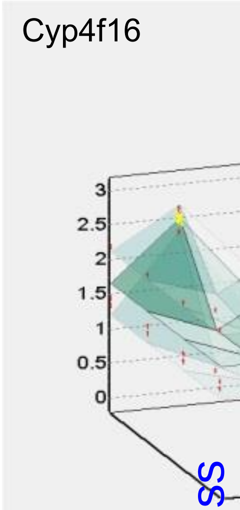
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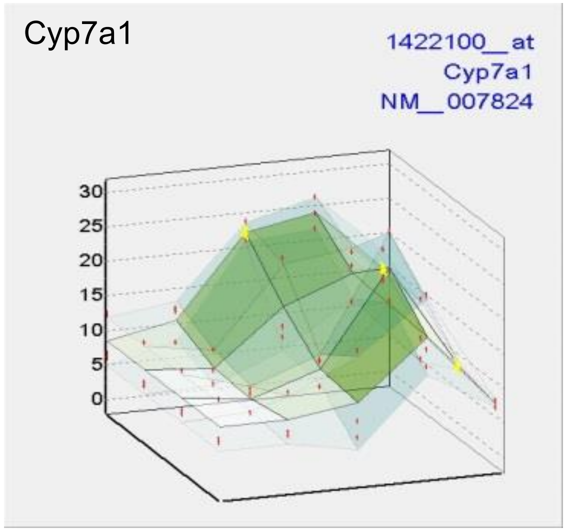
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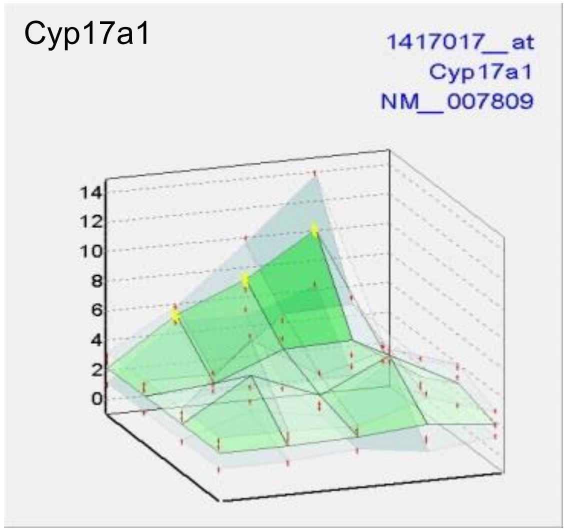
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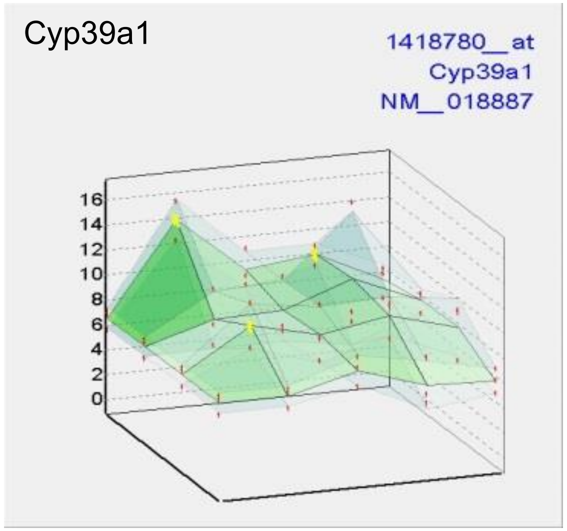
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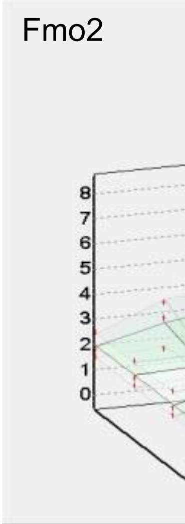

Fmo2

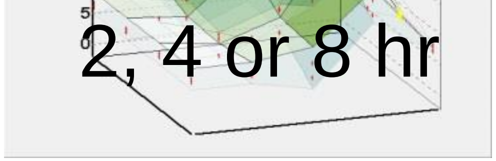
Fmo5

Nr1i2

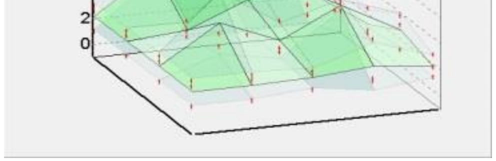
Junb

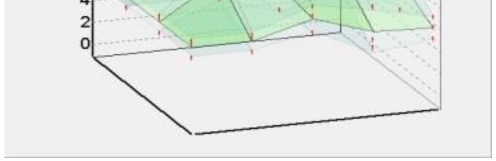
Junb
(Inverted)

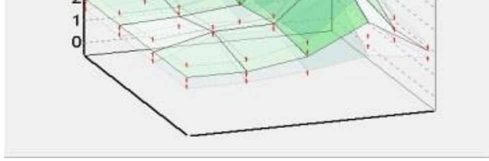
J Tox Sci, Vol. 38-4, 2013, in press

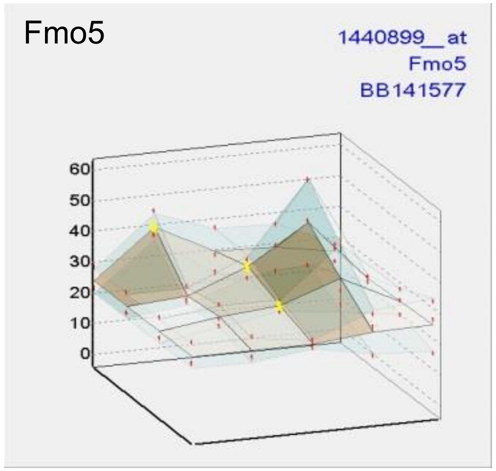


2, 4 or 8 hr





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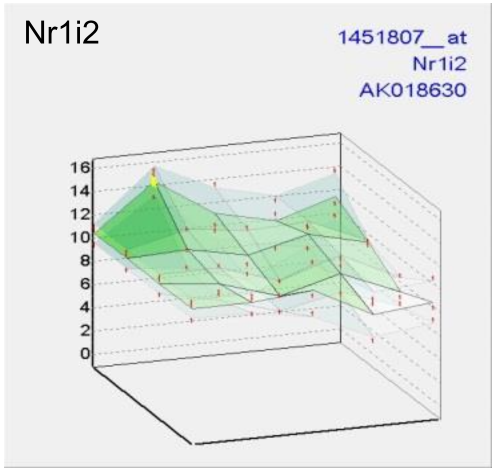
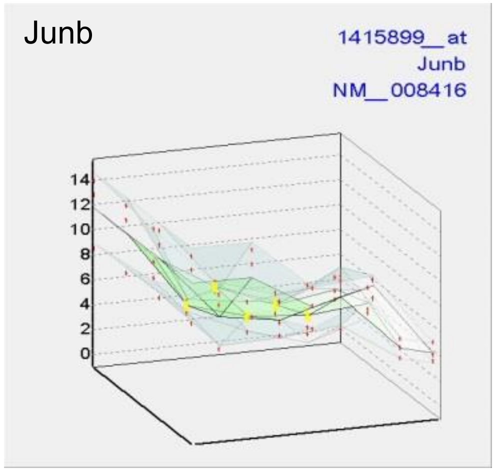
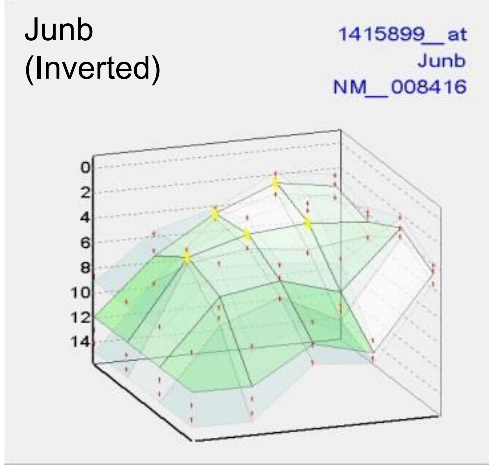
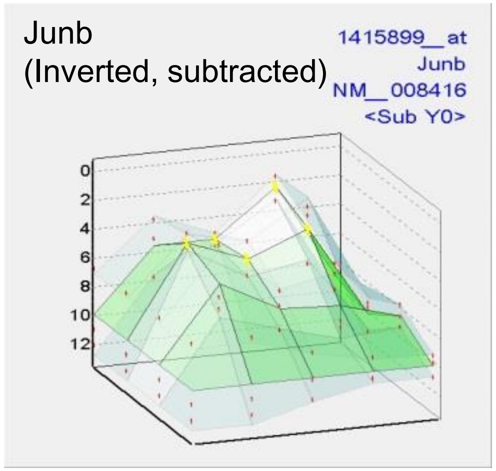
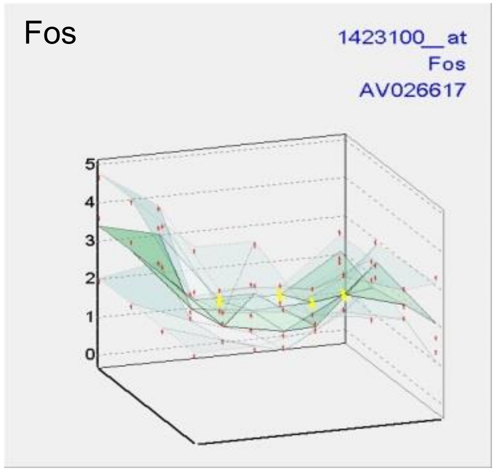

Nr1i2


Junb


Junb
(Inverted)


Junb
(Inverted, subtracted)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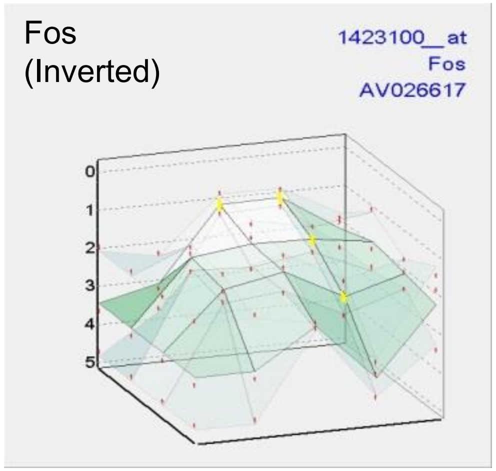
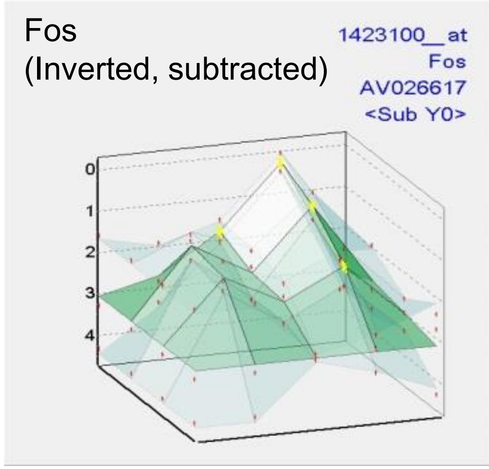
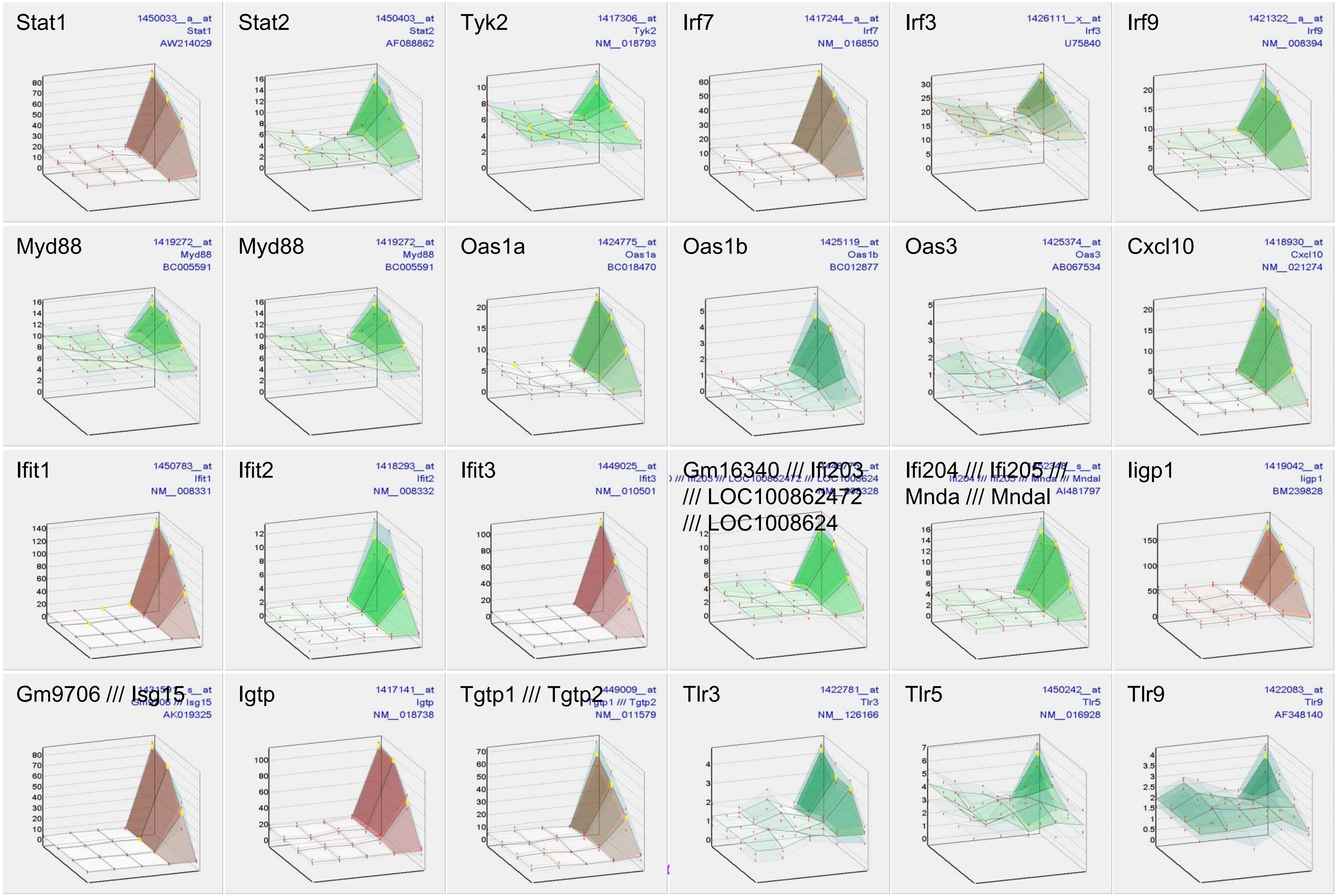

Fos
(Inverted)


Fos
(Inverted, subtracted)

J Tox Sci, Vol. 38-4, 2013, in press

Genes induced at 24 hr (unique to PCP)



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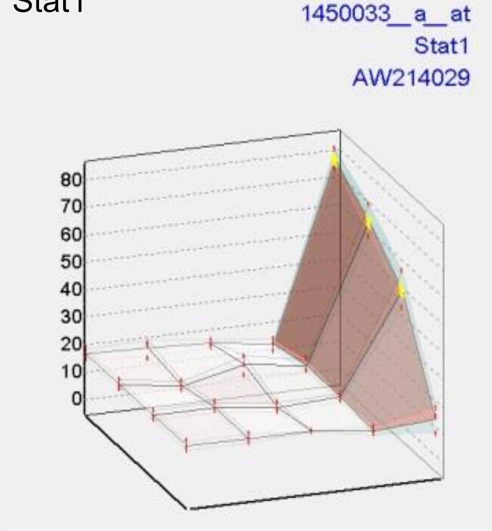
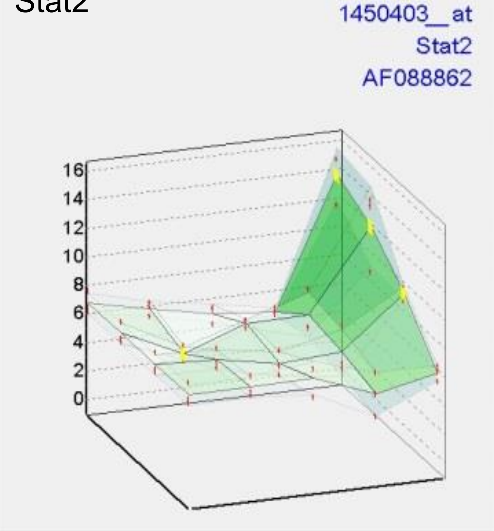
Genes induced at 24 hr (unique to PCP)

Stat1



Stat2



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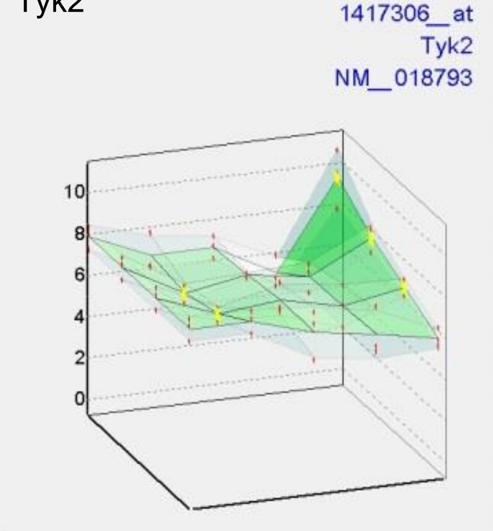
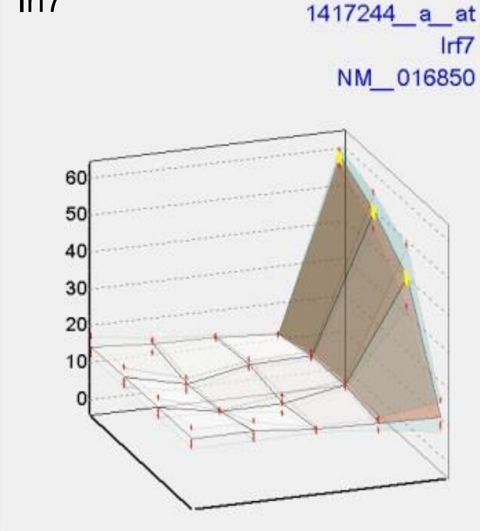
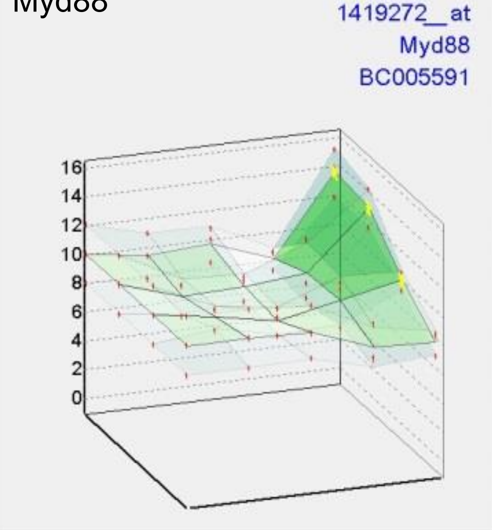


Irf7



Myd88



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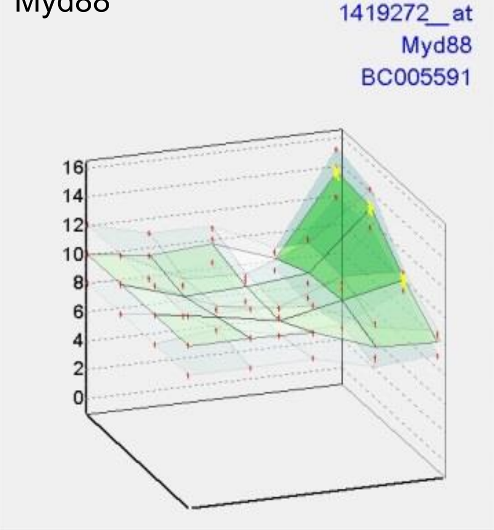

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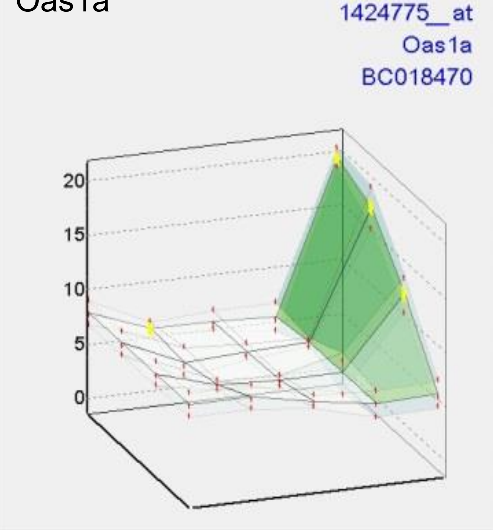

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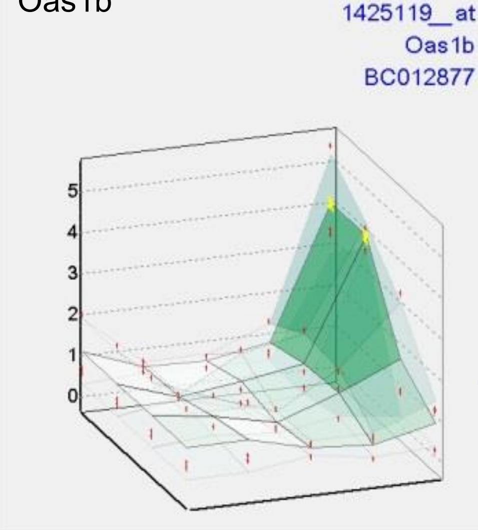
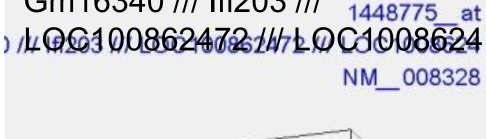


Ifit1

Ifit2

Ifit3

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



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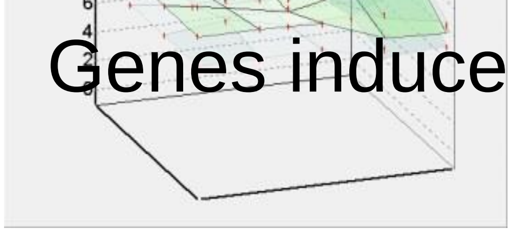
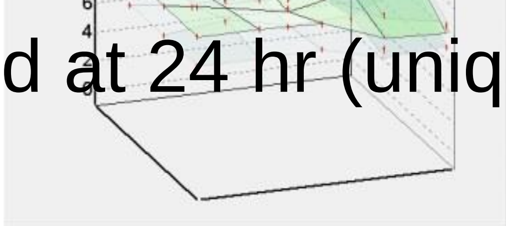
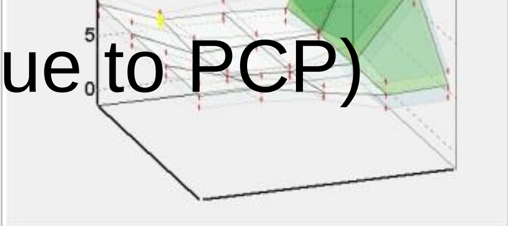
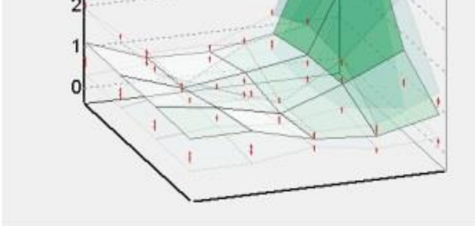
Genes induced at 24 hr (unique to PCP)

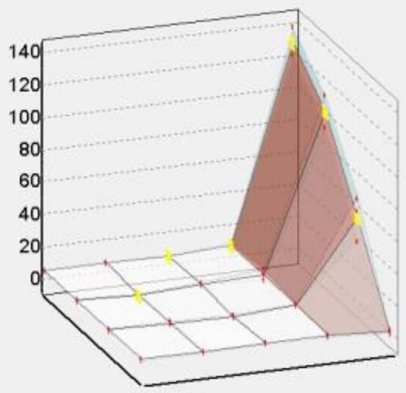








Ifit1



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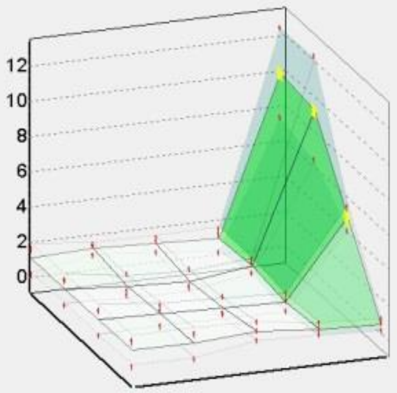
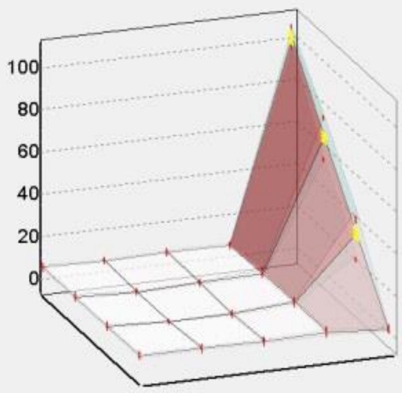


Ifit3



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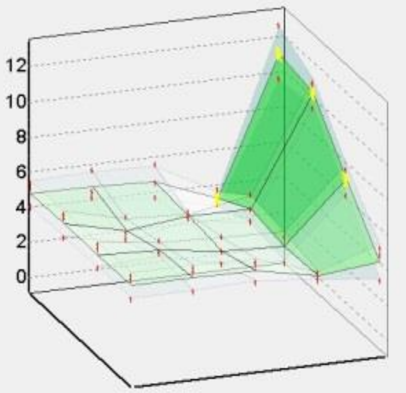
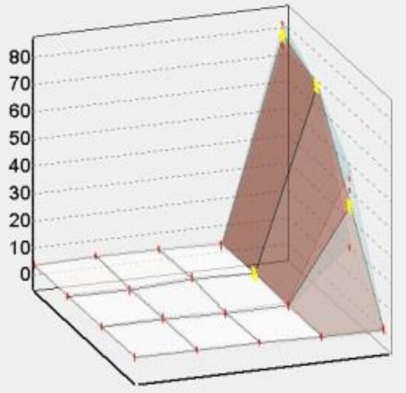
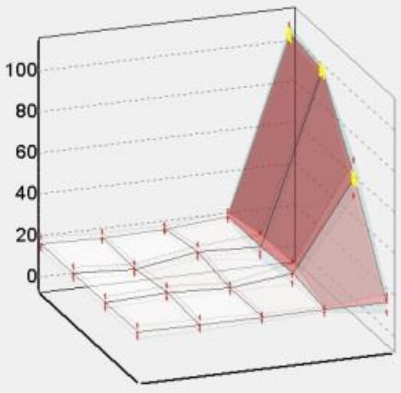


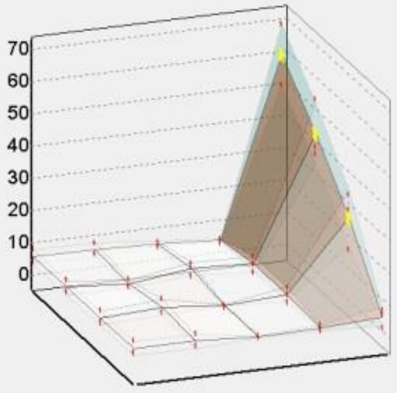
Gm9706 /// Isg15



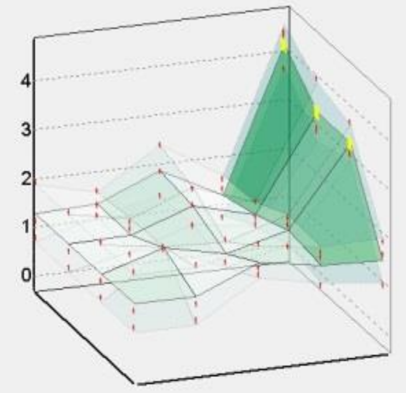
Igtp



Tgtp1 /// Tgtp2

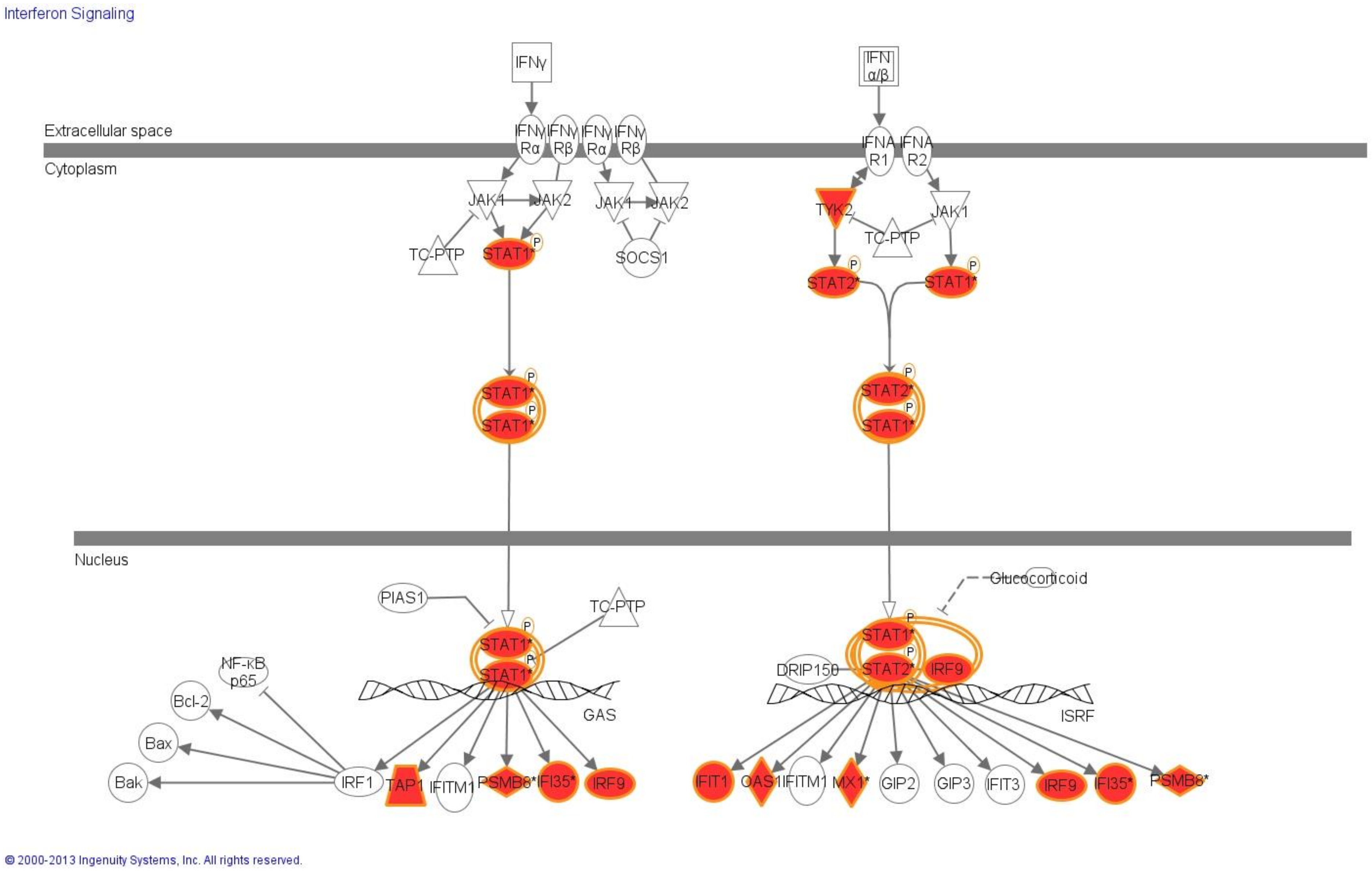


Tlr3



J Tox Sci, Vol. 38-4, 2013, in press

Interferon signaling (from IPA)


Interferon Signaling
IFNγ
IFN α/β
Extracellular space
Cytoplasm
IFNγ Rα
IFNγ Rβ
IFNγ Rα
IFNγ Rβ
IFNA R1
IFNA R2
JAK1
JAK2
JAK1
JAK2
TYK2
JAK1
TC-PTP
STAT1*
P
SOCS1
TC-PTP
STAT2*
STAT1*
STAT1*
STAT1*
STAT2*
STAT1*
Nucleus
PIAS1
TC-PTP
Glucocorticoid
STAT1*
STAT1*
STAT1*
STAT2*
IRF9
DRIP150
NF-κB p65
Bcl-2
Bax
Bak
GAS
ISRF
IRF1
TAP1
IFITM1
PSMB8*
IFI35*
IRF9
IFIT1
OAS1
IFITM1
MX1*
GIP2
GIP3
IFIT3
IRF9
IFI35*
PSMB8*

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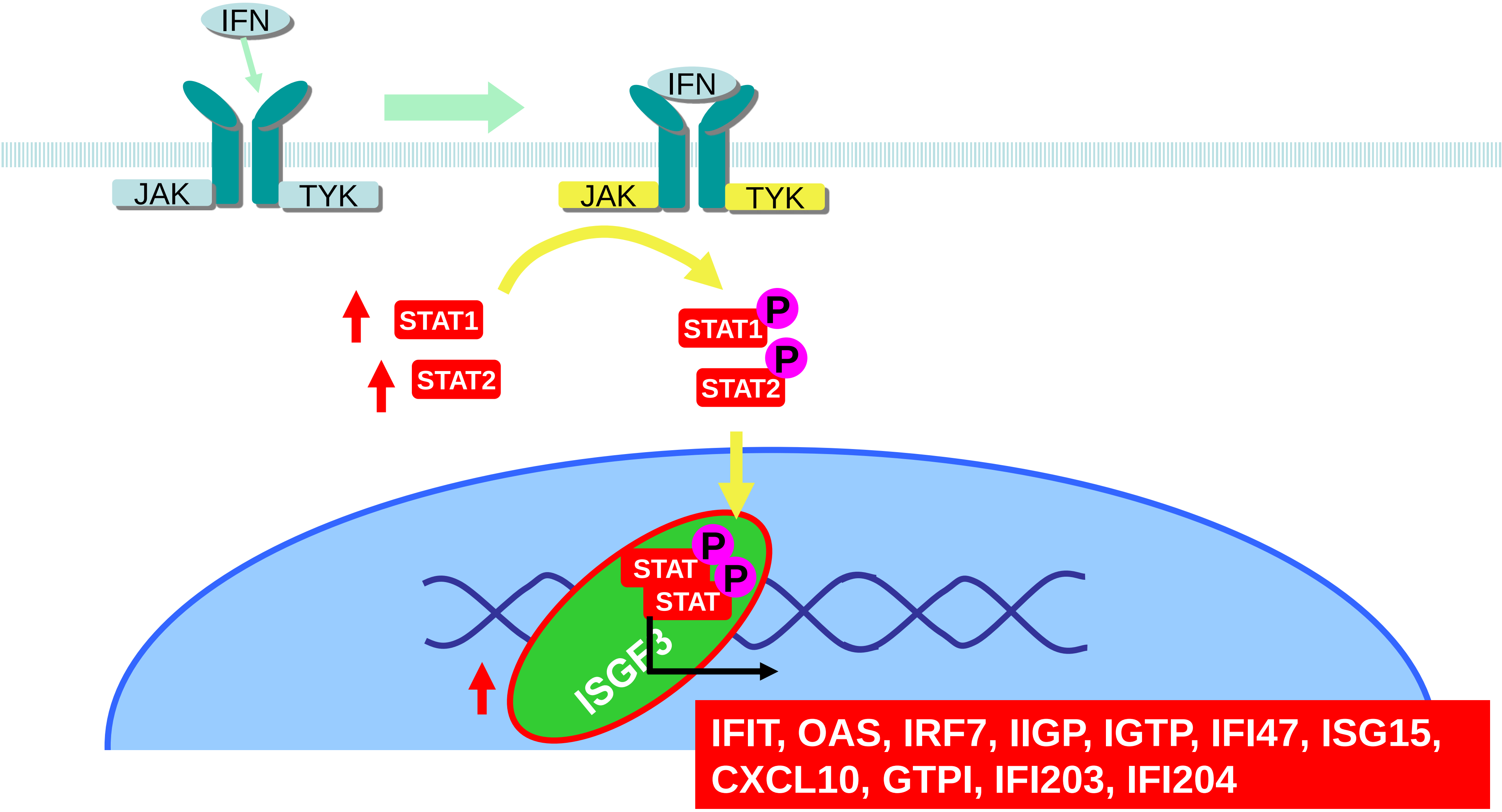
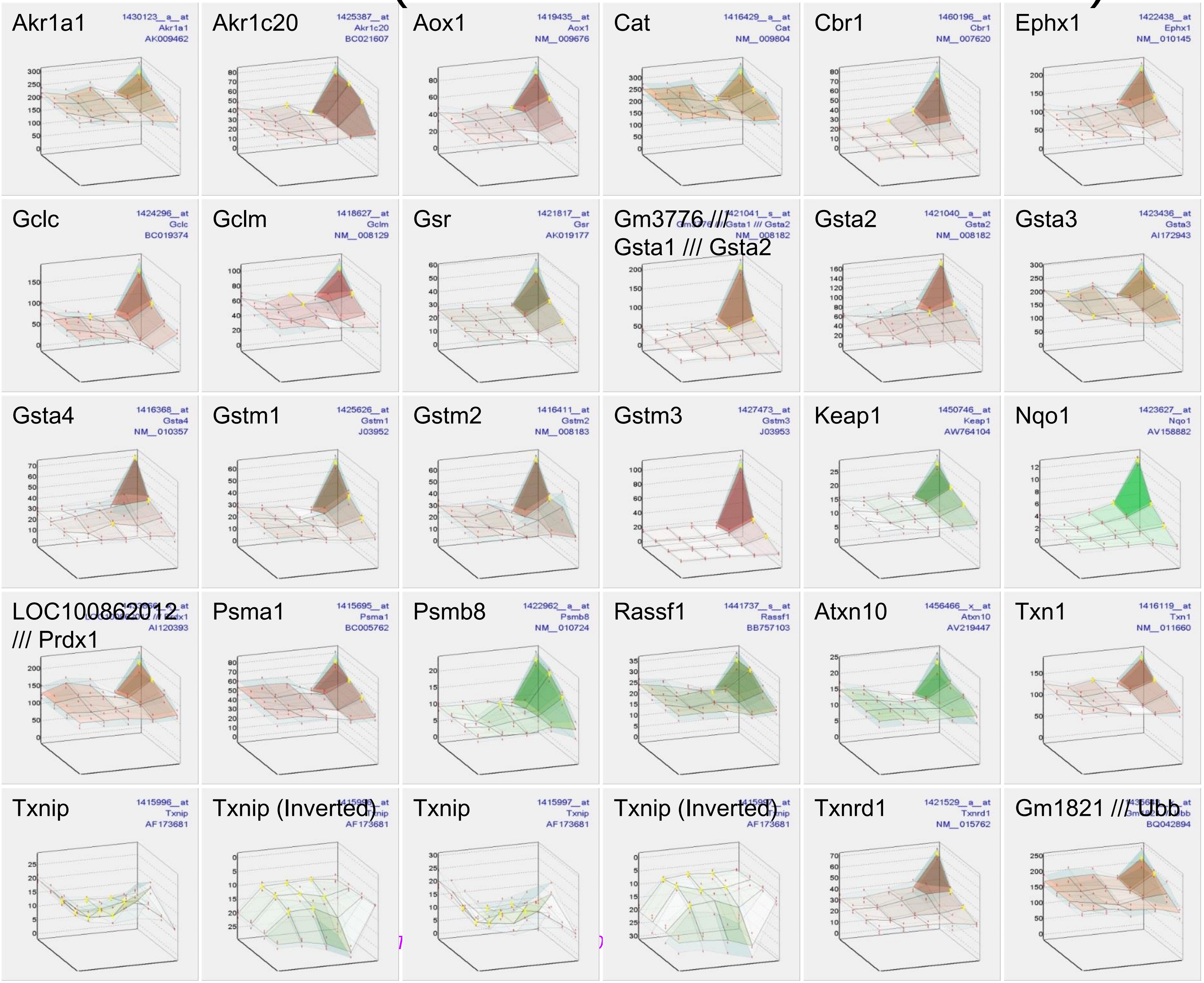

IFN
JAK
TYK
IFN
JAK
TYK
STAT1
STAT2
P
STAT1
P
STAT2
P
STAT
P
STAT
ISGF3

IFIT, OAS, IRF7, IIGP, IGTP, IFI47, ISG15, CXCL10, GTPI, IFI203, IFI204

Genes induced at 24 hr (Common to other chemicals)

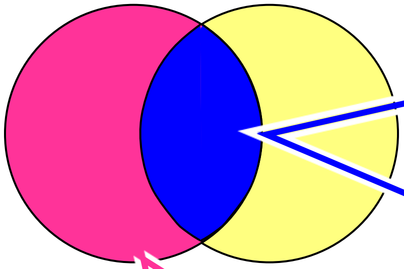


J Tox Sci, Vol. 38-4, 2013, in press

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

PCP

Sum Set of Other chemicals in Percellome DB

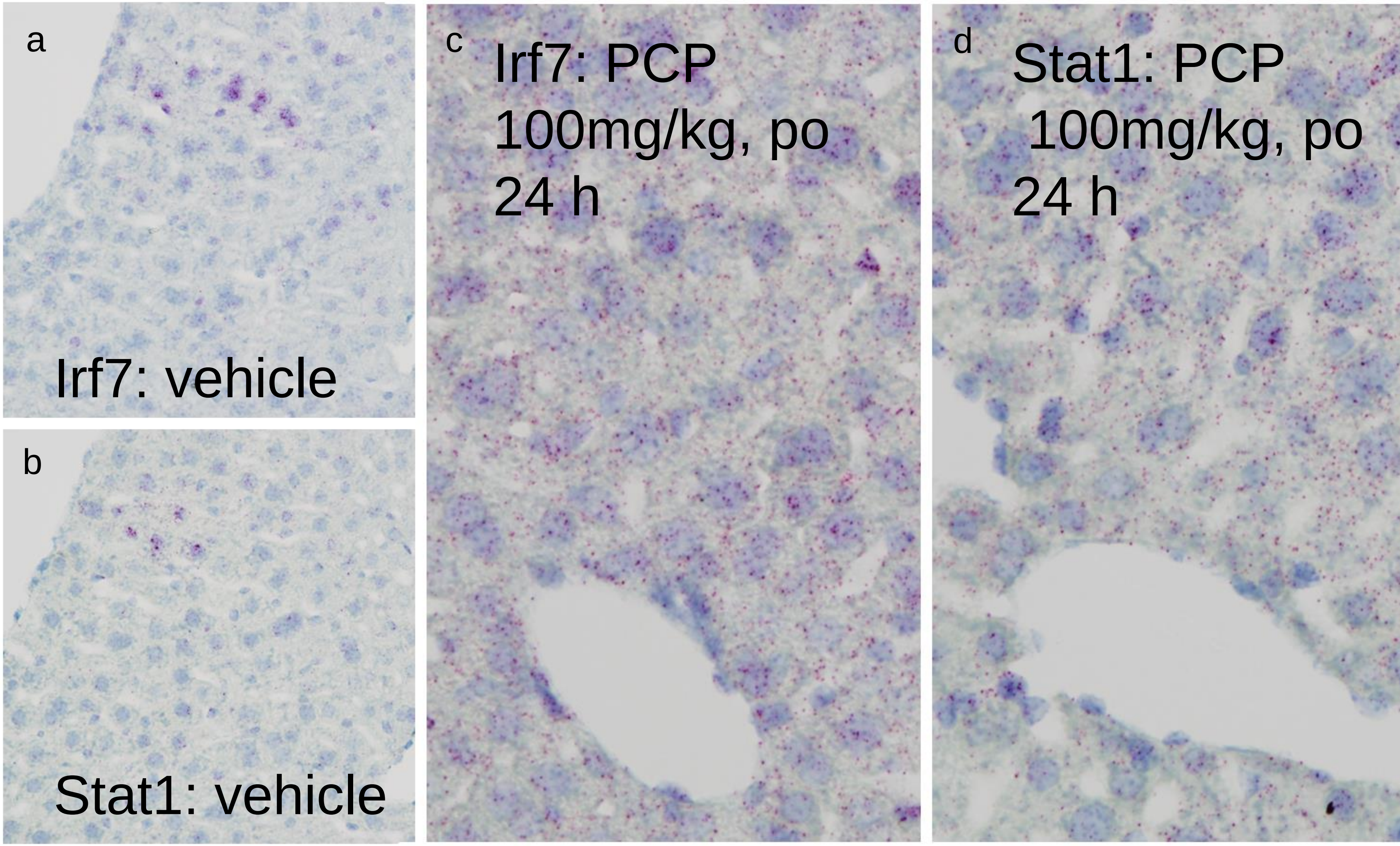


NRF2-mediated Oxidative Stress Response	6.3×10^{-18}
Protein Ubiquitination Pathway	3.0×10^{-14}
Xenobiotic Metabolism Signaling	2.2×10^{-05}
Cell Cycle Control of Chromosomal Replication	5.7×10^{-05}
Glutathione-mediated Detoxification	7.1×10^{-05}

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}

J Tox Sci, Vol. 38-4, 2013, in press

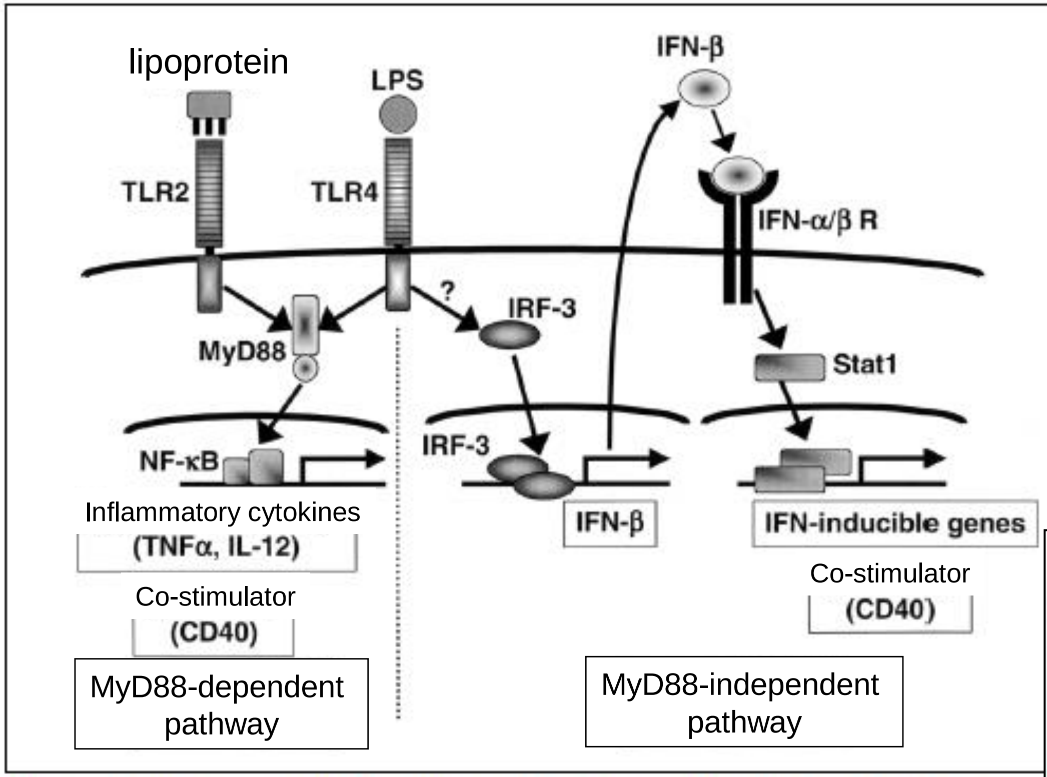
In situ hybridization; QuantiGeneViewRNA ISH Tissue Assay kit (Affymetrix, Inc.)

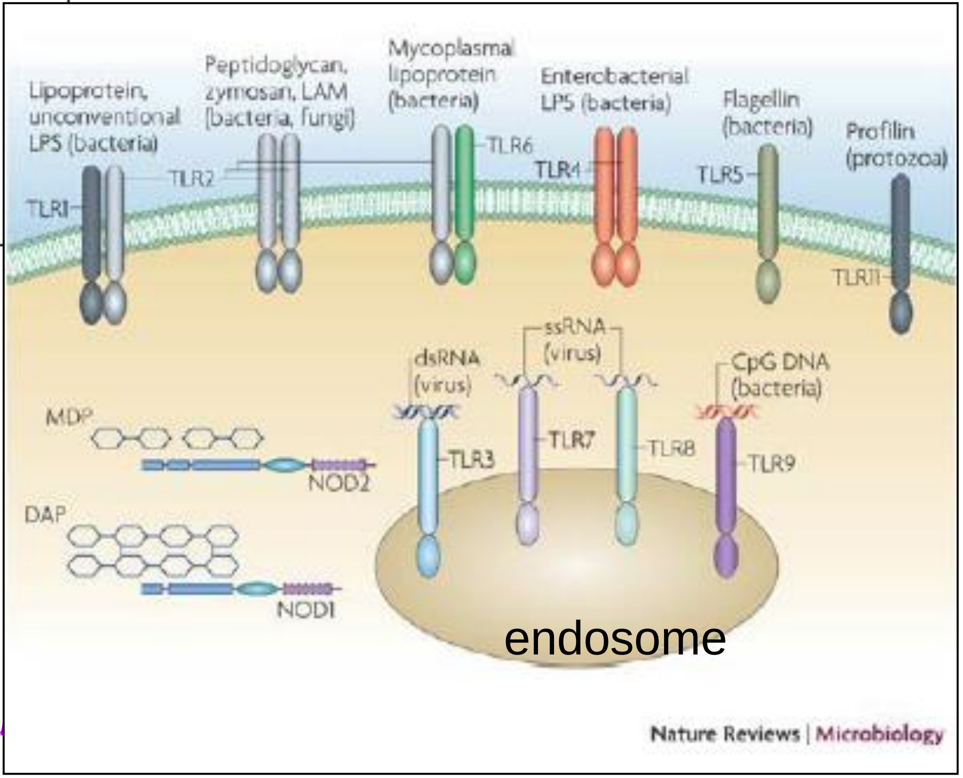

a
Irf7: vehicle
b
Stat1: vehicle
c
Irf7: PCP
100mg/kg, po
24 h
d
Stat1: PCP
100mg/kg, po
24 h

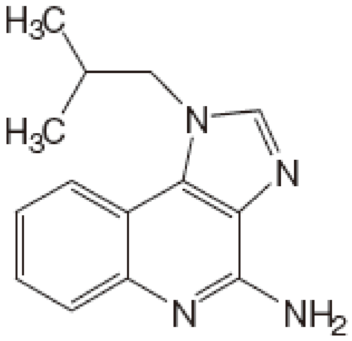
J Tox Sci, Vol. 38-4, 2013, in press

From: Inflammation and Regeneration Vol.23 No.4 JULY 2003

By S. Akira


lipoprotein
LPS
TLR2
TLR4
IFN-β
IFN-α/β R
?
IRF-3
MyD88
Stat1
NF-κB
IRF-3
Inflammatory cytokines
(TNFα, IL-12)
IFN-β
IFN-inducible genes
Co-stimulator
(CD40)
Co-stimulator
(CD40)
MyD88-dependent pathway
MyD88-independent pathway


Lipoprotein, unconventional LPS (bacteria)
Peptidoglycan, zymosan, LAM (bacteria, fungi)
Mycoplasma lipoprotein (bacteria)
Enterobacterial LPS (bacteria)
Flagellin (bacteria)
Profilin (protozoa)
TLR1
TLR2
TLR6
TLR4
TLR5
TLR11
ssRNA (virus)
dsRNA (virus)
CpG DNA (bacteria)
MDP
TLR3
TLR7
TLR8
TLR9
NOD2
DAP
NOD1
endosome
Nature Reviews | Microbiology



imiquimod

Neuroscience. 2012 Jan 10;201:166-83. doi: 10.1016/j.neuroscience.2011.11.013. Epub 2011 Nov 12.

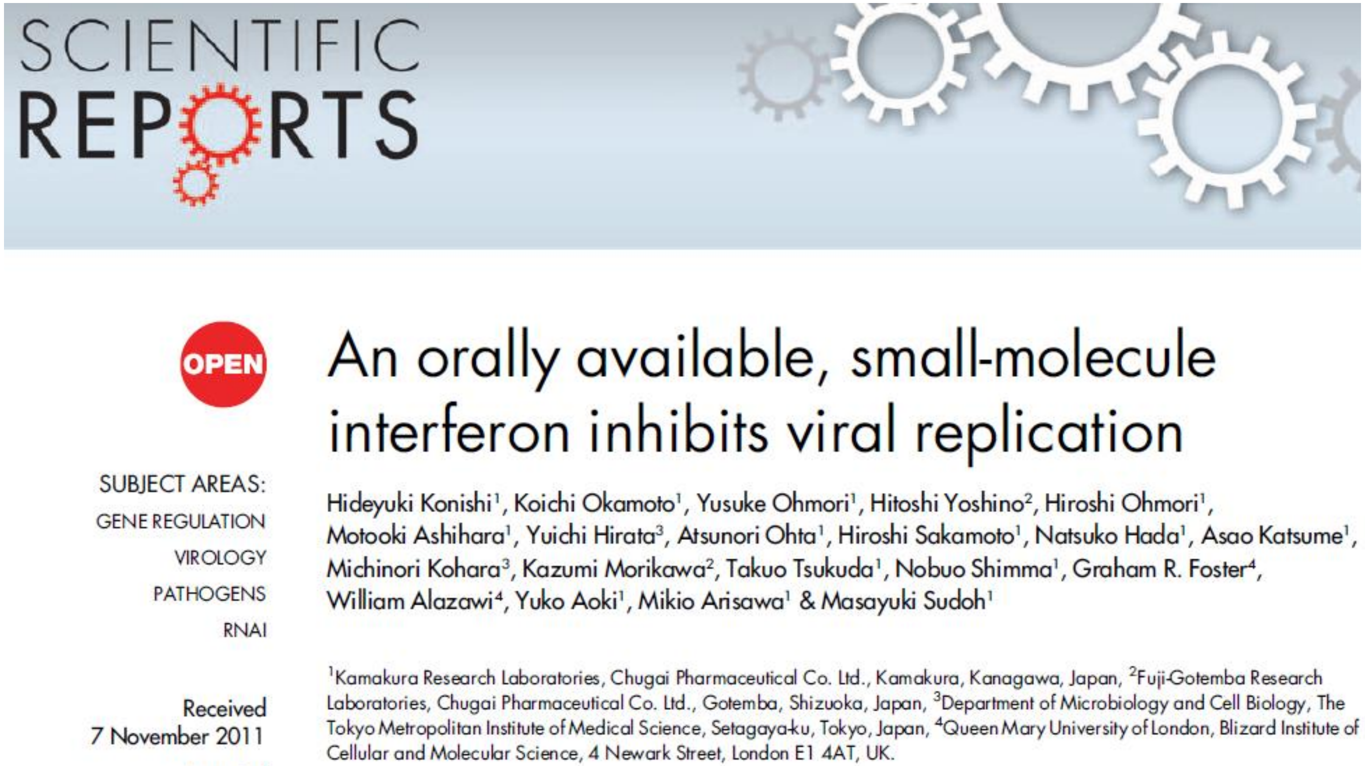
Fever, sickness behavior, and expression of inflammatory genes in the hypothalamus after systemic and localized subcutaneous stimulation of rats with the Toll-like receptor 7 agonist imiquimod.

Damm J, Wiegand F, Harden LM, Gerstberger R, Rummel C, Roth J.

Institut für Veterinär-Physiologie und -Biochemie, Justus-Liebig-Universität Giessen, Frankfurter Strasse 100, D-35392 Giessen, Germany.

Abstract

The Toll-like receptor 7 (TLR7) agonist imiquimod is used for topical treatment of skin cancers. We studied the consequences of injections of imiquimod into a subcutaneous (s.c.) air pouch or of intraperitoneal (i.p.) injections on the manifestation of fever, sickness behavior, and the peripheral and brain-intrinsic induction of a variety of inflammatory molecules. Rats were given imiqimod s.c. or i.p. (1 or 5 mg/kg). Body temperature, motor activity, and food and water intake were recorded by telemetric devices. Peripheral and brain-intrinsic induction of inflammatory mediators was analyzed by real-time polymerase chain reaction (RT-PCR), bioassays, enzyme-linked immunosorbent assays (ELISAs), and immunohistochemistry. Imiquimod is the first TLR-agonist to produce more potent effects with s.c. than i.p. administration. Peripheral induction of interferons (IFNs) and putative circulating pyrogens corresponded to the magnitude of the illness responses. In the brain, an expression of cytokines (TNFα, IL-1β, and IL-6) and inducible forms of enzymes for prostaglandin E2 synthesis (COX-2 and mPGES) occurred, which was accompanied by a moderate activation of the transcription factors NFκB and STAT3, and a strong activation of the transcription factor NF-IL6, in cells of specific areas with an open blood-brain barrier. These inflammatory responses noted within the brain were more marked after s.c. administration, than i.p. administration of imiquimod. At a dose of 5 mg/kg, imiquimod causes rather moderate brain-inflammatory responses, which are related to peripheral IFN-expression and possibly mediated by brain-intrinsic activation of NF-IL6 and induction of a proinflammatory cocktail. The lack of a



SCIENTIFIC REPORTS

OPEN

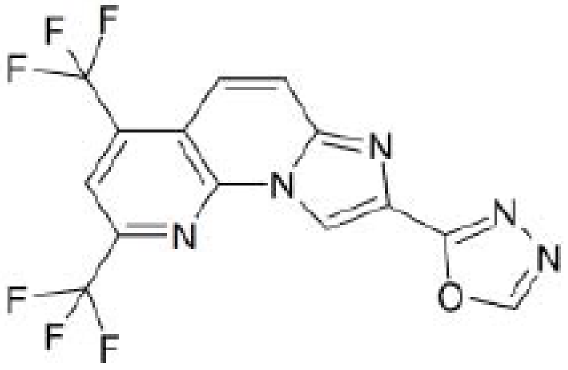
An orally available, small-molecule interferon inhibits viral replication

SUBJECT AREAS:
GENE REGULATION
VIROLOGY
PATHOGENS
RNAI

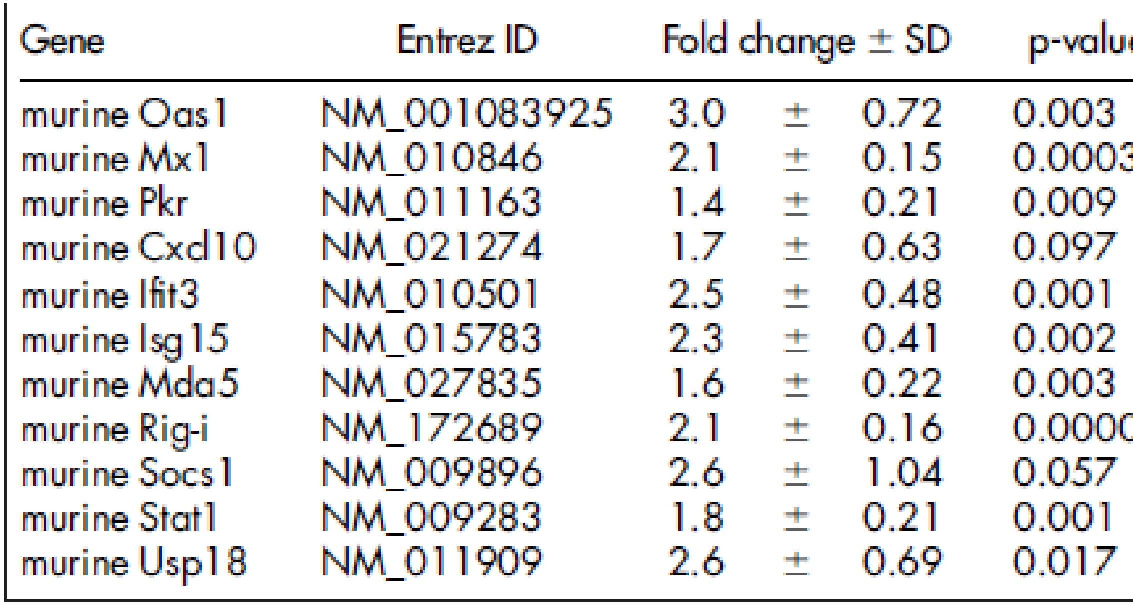
Hideyuki Konishi[1], Koichi Okamoto[1], Yusuke Ohmori[1], Hitoshi Yoshino[2], Hiroshi Ohmori[1], Motooki Ashihara[1], Yuichi Hirata[3], Atsunori Ohta[1], Hiroshi Sakamoto[1], Natsuko Hada[1], Asao Katsume[1], Michinori Kohara[3], Kazumi Morikawa[2], Takuo Tsukuda[1], Nobuo Shimma[1], Graham R. Foster[4], William Alazawi[4], Yuko Aoki[1], Mikio Arisawa[1] & Masayuki Sudoh[1]

[1]Kamakura Research Laboratories, Chugai Pharmaceutical Co. Ltd., Kamakura, Kanagawa, Japan, [2]Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd., Gotemba, Shizuoka, Japan, [3]Department of Microbiology and Cell Biology, The Tokyo Metropolitan Institute of Medical Science, Setagaya-ku, Tokyo, Japan, [4]Queen Mary University of London, Blizard Institute of Cellular and Molecular Science, 4 Newark Street, London E1 4AT, UK.

Received
7 November 2011
Accepted



RO4948191



Gene	Entrez ID	Fold change ± SD	p-valu
murine Oas1	NM_001083925	3.0 ± 0.72	0.003
murine Mx1	NM_010846	2.1 ± 0.15	0.0003
murine Pkr	NM_011163	1.4 ± 0.21	0.009
murine Cxcl10	NM_021274	1.7 ± 0.63	0.097
murine Ifit3	NM_010501	2.5 ± 0.48	0.001
murine Isg15	NM_015783	2.3 ± 0.41	0.002
murine Mda5	NM_027835	1.6 ± 0.22	0.003
murine Rig-i	NM_172689	2.1 ± 0.16	0.0000
murine Socs1	NM_009896	2.6 ± 1.04	0.057
murine Stat1	NM_009283	1.8 ± 0.21	0.001
murine Usp18	NM_011909	2.6 ± 0.69	0.017

ICTX

TOXICOLOGICAL SCIENCES 125(1), 144–156 (2012)
doi:10.1093/toxsci/kfr283
Advance Access publication October 20, 2011

The Dysregulation of the Monocyte/Macrophage Effector Function Induced by Isopropanol Is Mediated by the Defective Activation of Distinct Members of the AP-1 Family of Transcription Factors

Damien Carignan,[1] Olivier Désy,[1] and Pedro O. de Campos-Lima[2]

Laval University Cancer Research Center, Quebec City, Quebec G1R 2J6, Canada

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[2]To whom correspondence should be addressed at Laval University Cancer Research Center, McMahon Street 9, Quebec City, Quebec G1R 2J6, Canada.
Fax: +1-418-691-5439. E-mail: pedro.campos-lima@crhdq.ulaval.ca.

Received July 18, 2011; accepted October 11, 2011

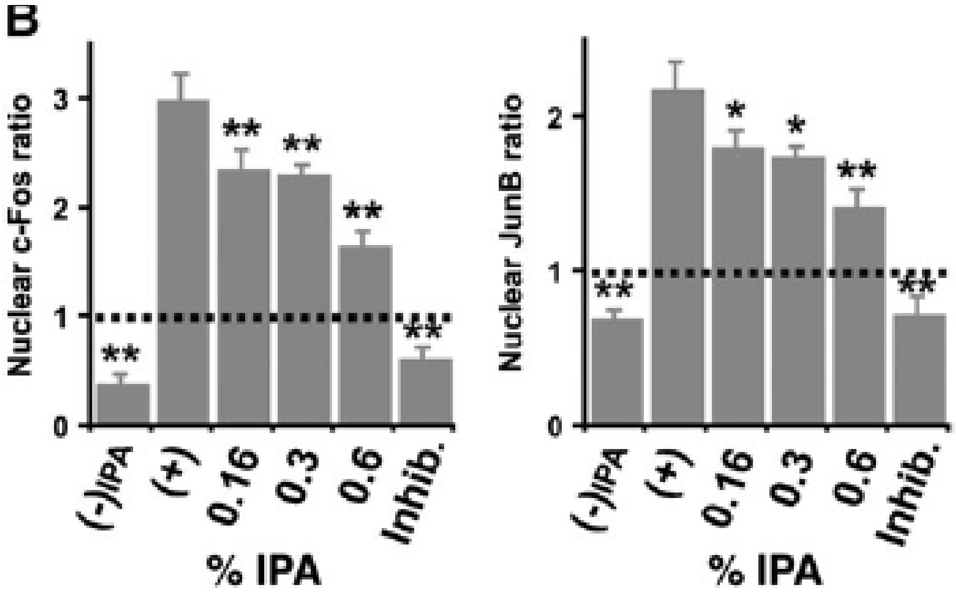
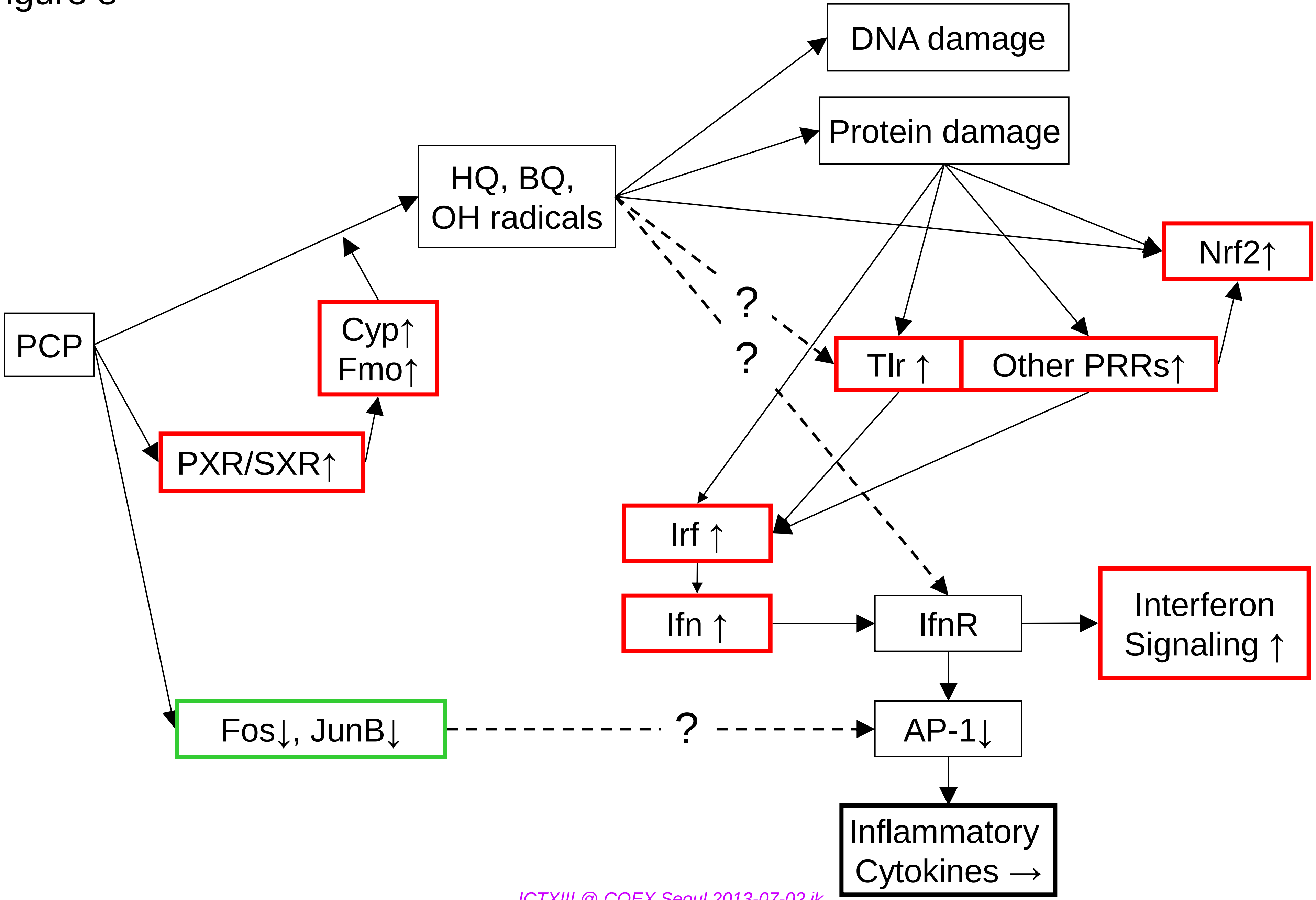
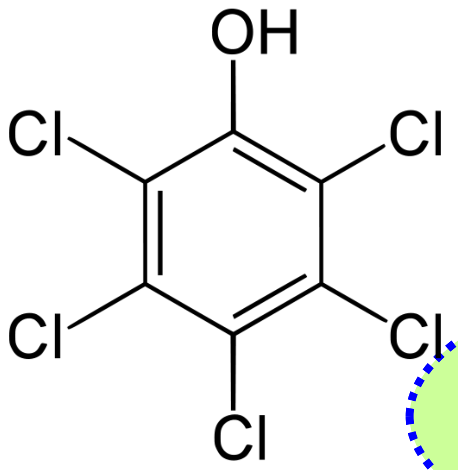


FIG. 4. Isopropanol induces a selective defect in the MAPK signaling cascade and alters the activation of discrete AP-1 family members. (A)

Figure 8


DNA damage
Protein damage
HQ, BQ,
OH radicals
PCP
Cyp↑
Fmo↑
Nrf2↑
?
?
Tlr ↑
Other PRRs↑
PXR/SXR↑
Irf ↑
Ifn ↑
IfnR
Interferon
Signaling ↑
Fos↓, JunB↓
?
AP-1↓
Inflammatory
Cytokines →

Pentachlorophenol (PCP)



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

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

J Tox Sci, Vol. 38-4, 2013, in press

Summary

- Hyperthermia and other functional symptoms have been explained by the uncoupling of oxidative phosphorylation in mitochondria.
- Our data suggested that those functional symptoms can be explained by the activation of interferon signaling.
- These symptoms are similar to that of Influenza
- Two possible mechanisms were discussed; indirect mechanism via the PRR system, and direct stimulation of the TLR(s) or interferon receptor(s).
- Further study is needed to clarify the molecular mechanisms.

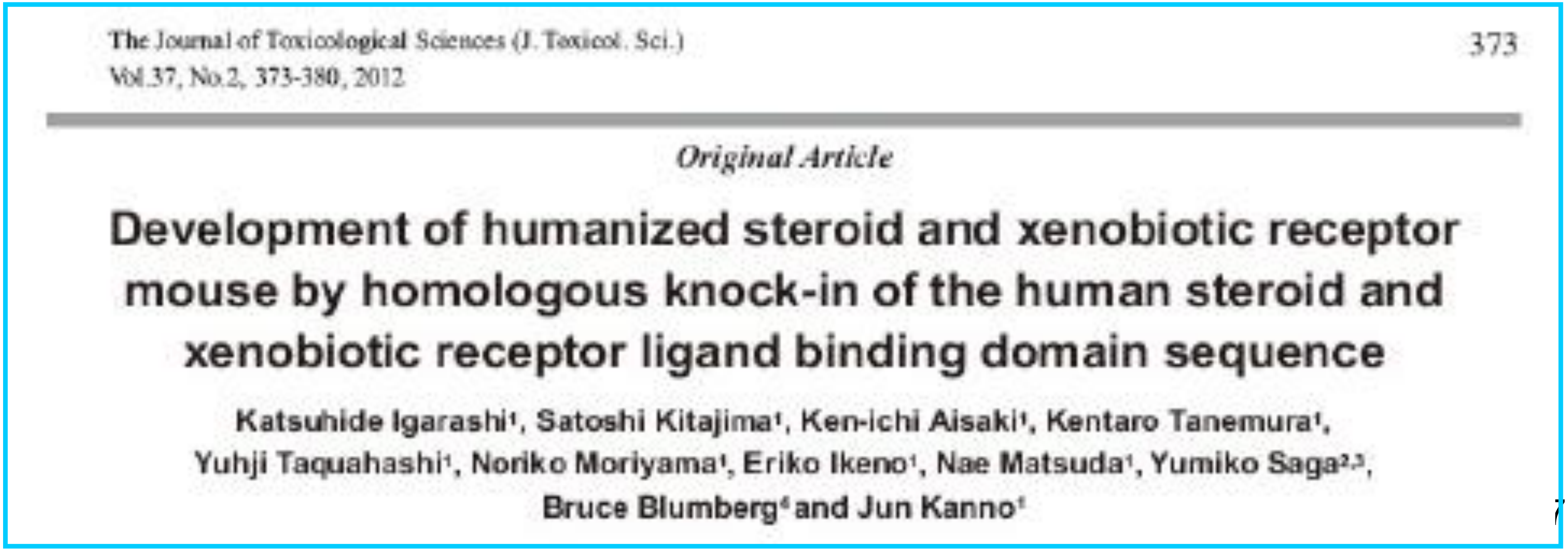
Gene Knock-out Mouse

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

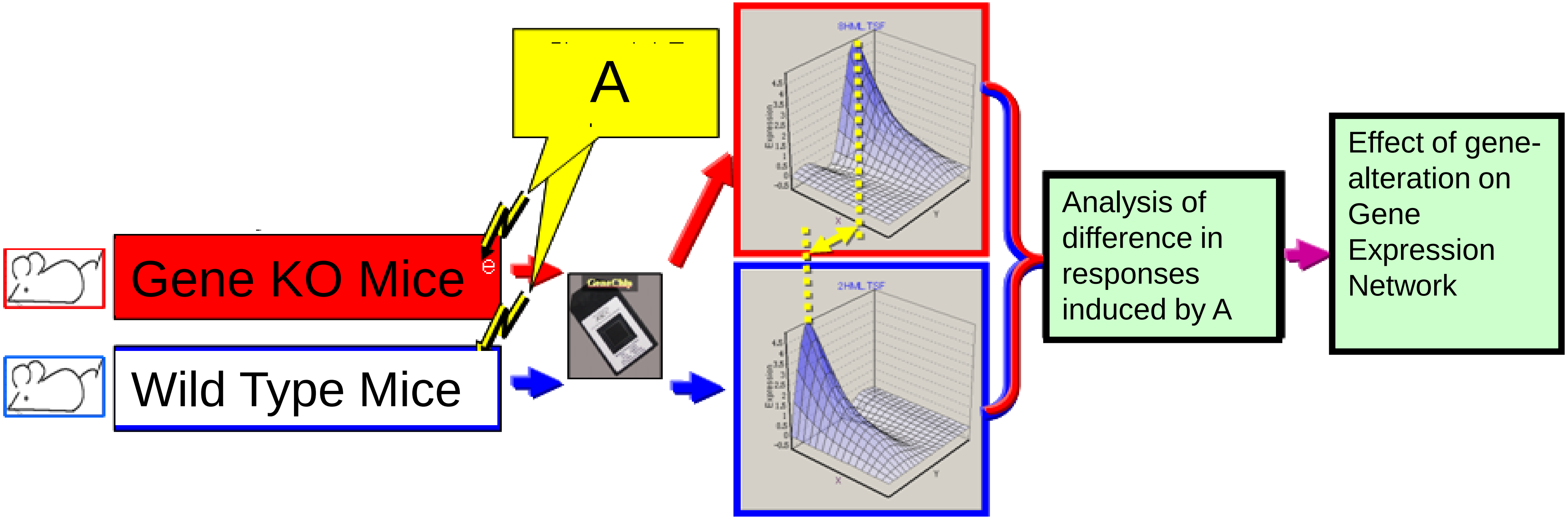
373

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


GeneChip
Expression
X
Y

A

Gene KO Mice

Wild Type Mice

Analysis of difference in responses induced by A

Effect of gene-alteration on Gene Expression Network

Unpublished data

- AhRKO (aryl hydrocarbon receptor KO)

  3-MC (3-methylcholanthrene)

  TCDD (2,3,7,8-tetracholorodibenzo-*p*-dioxin)

Unpublished data

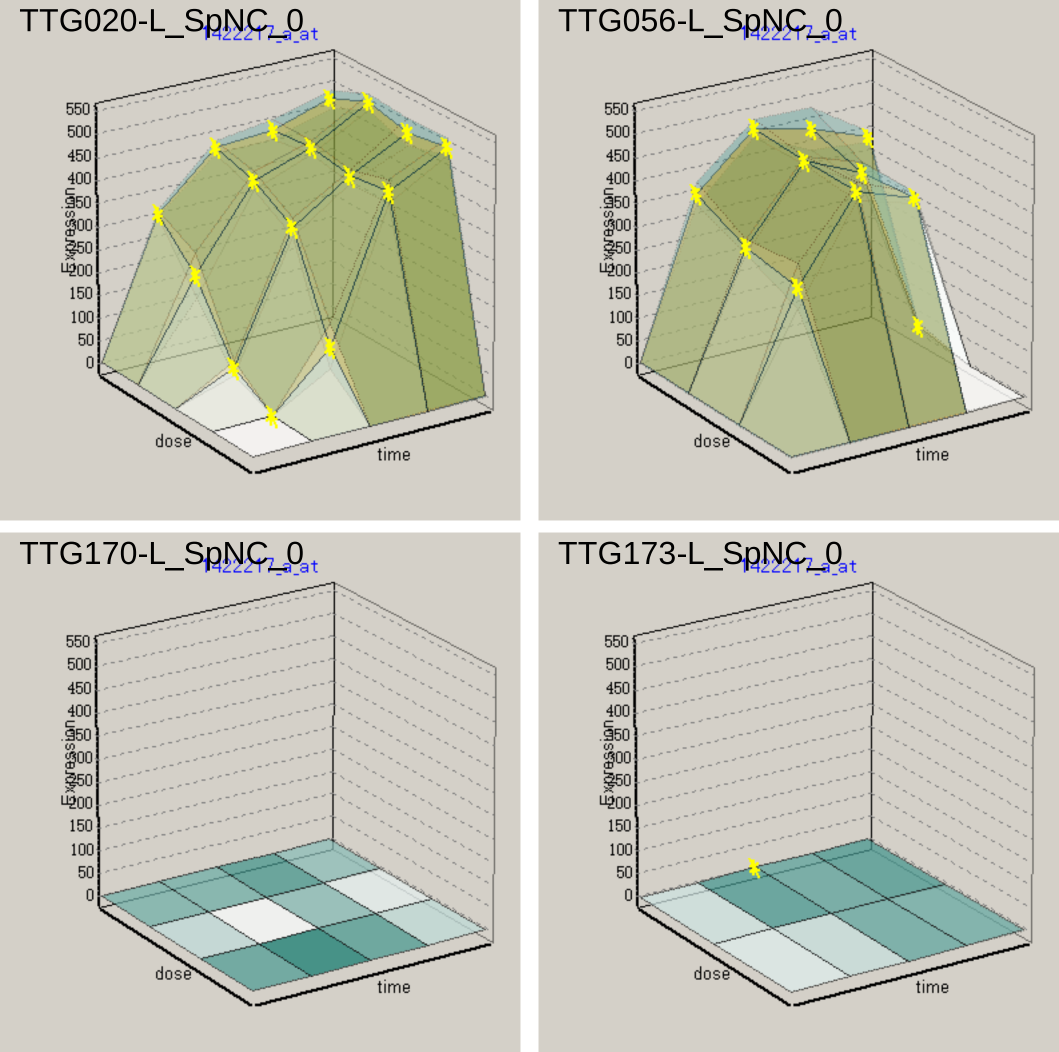
Cyp1a1
NM_009992
cytochrome P450, family 1, subfamily a, polypeptide 1

TCDD

3-MC

Wild mouse

AhR KO mouse



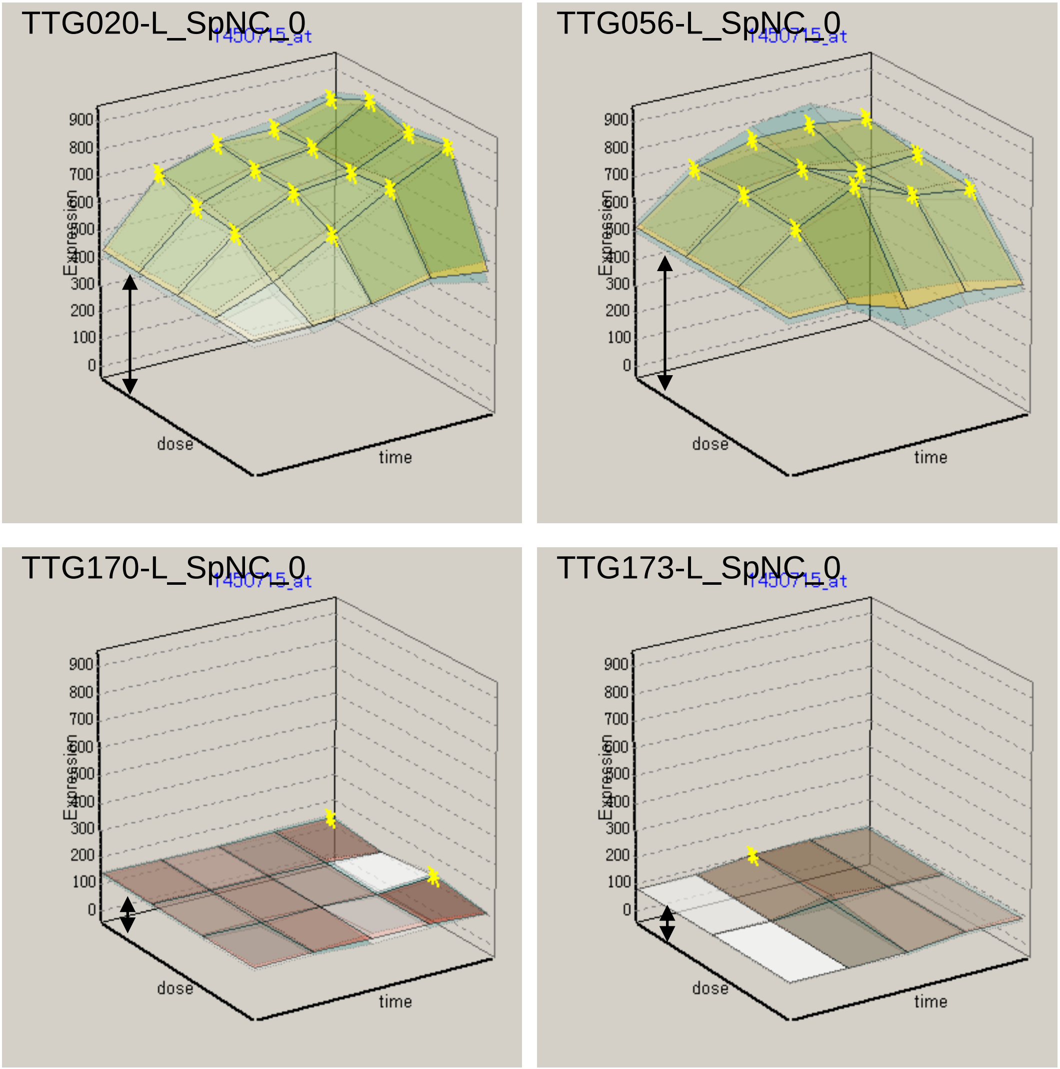
*:T-test $p<0.05$ compared to concurrent vehicle

Unpublished data

TCDD 3-MC

Wild type mouse

AhR KO mouse



Cyp1a2
NM_009993
cytochrome P450, family 1, subfamily a, polypeptide 2

Unpublished data

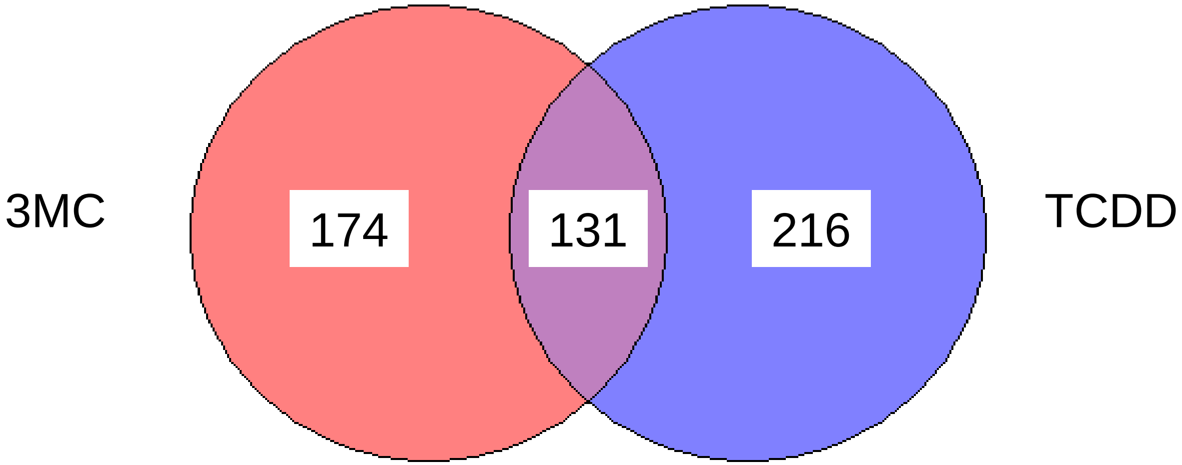
AhR-related studies

- AhRKO 3-MC = almost Null response
- AhRKO TCDD = almost Null response
  - 3-MC dose not get metabolized by KO mice

- Wild mouse
  - TCDD induces pure AhR singal network
  - 3-MC induces pure AhR + metabolite signal network

- [Wild 3-MC] - [Wild TCDD]
  = 3-MC metabolite signal network

[Wild 3-MC] - [Wild TCDD]



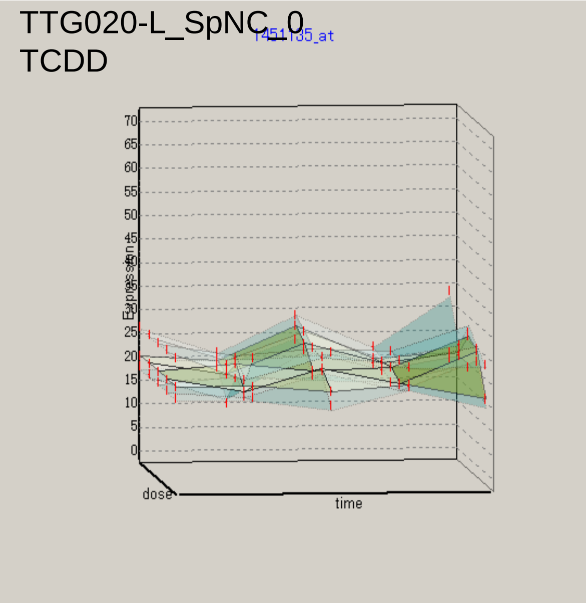
ICTXIII @ COEX Seoul 2013-07-02 jk

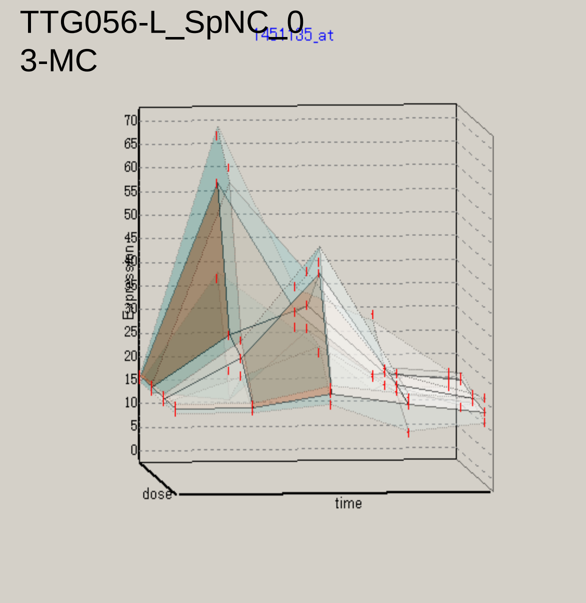
Unpublished data

TCDD

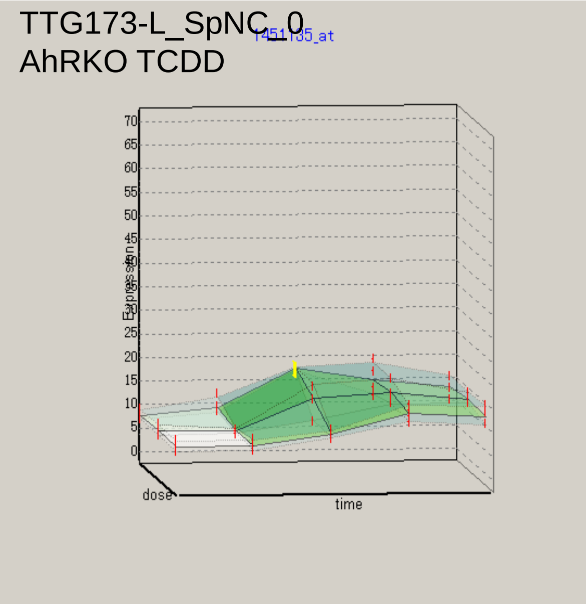
3-MC

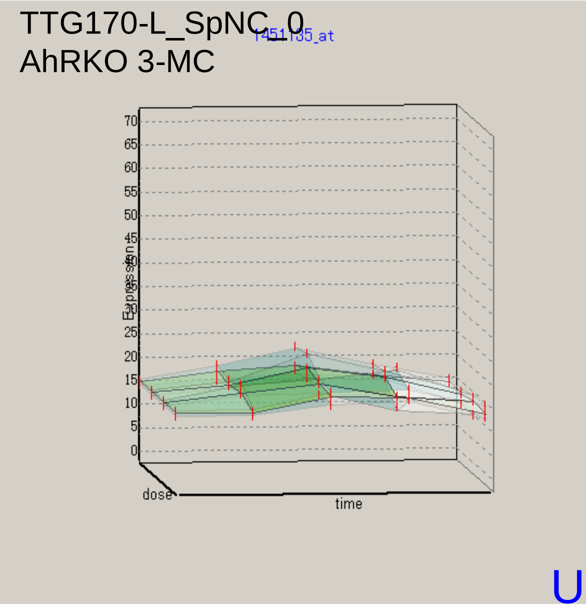
Wild


TTG020-L_SpNC_0
TCDD


TTG056-L_SpNC_0
3-MC

AhR KO


TTG173-L_SpNC_0
AhRKO TCDD


TTG170-L_SpNC_0
AhRKO 3-MC

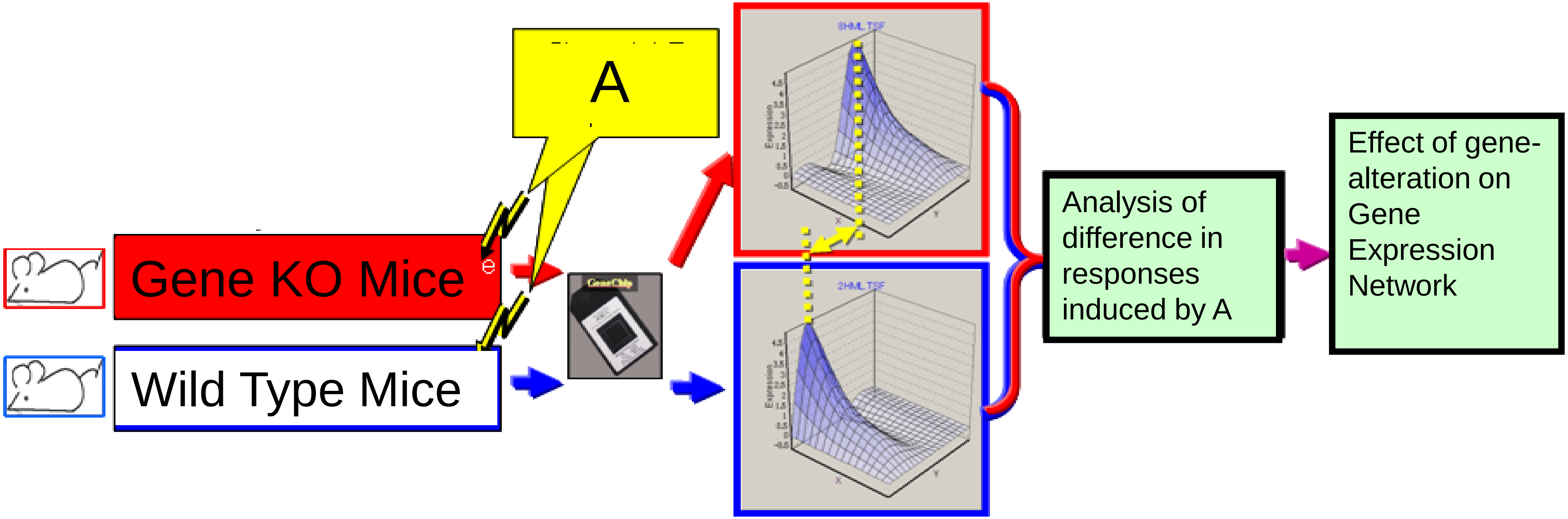
Gtf2b
1451135_at
general transcription
factor IIB

Unpublished data

New Concept of Repeated Dose

Gene Knockout Mice

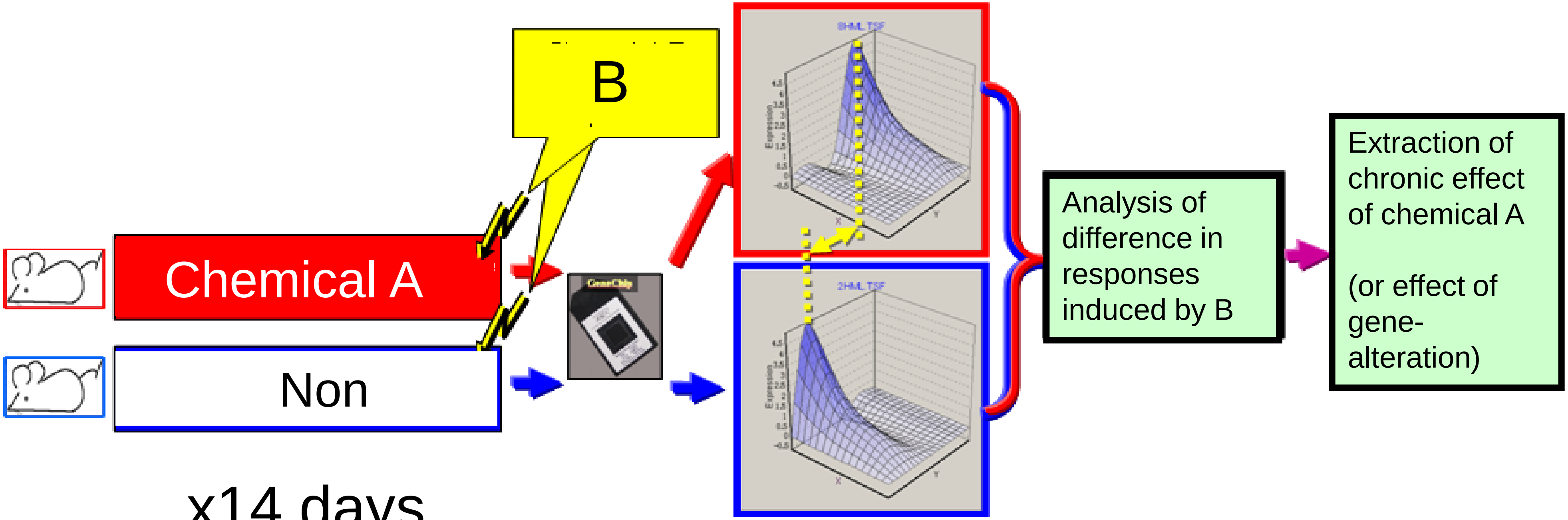
New Concept of Repeated Dose


A
Gene KO Mice
Wild Type Mice
GeneChip
8HML TSF
2HML TSF
Expression
X
Y

Analysis of difference in responses induced by A

Effect of gene-alteration on Gene Expression Network

New Concept of Repeated Dose



B

Chemical A

Non

x14 days

Analysis of difference in responses induced by B

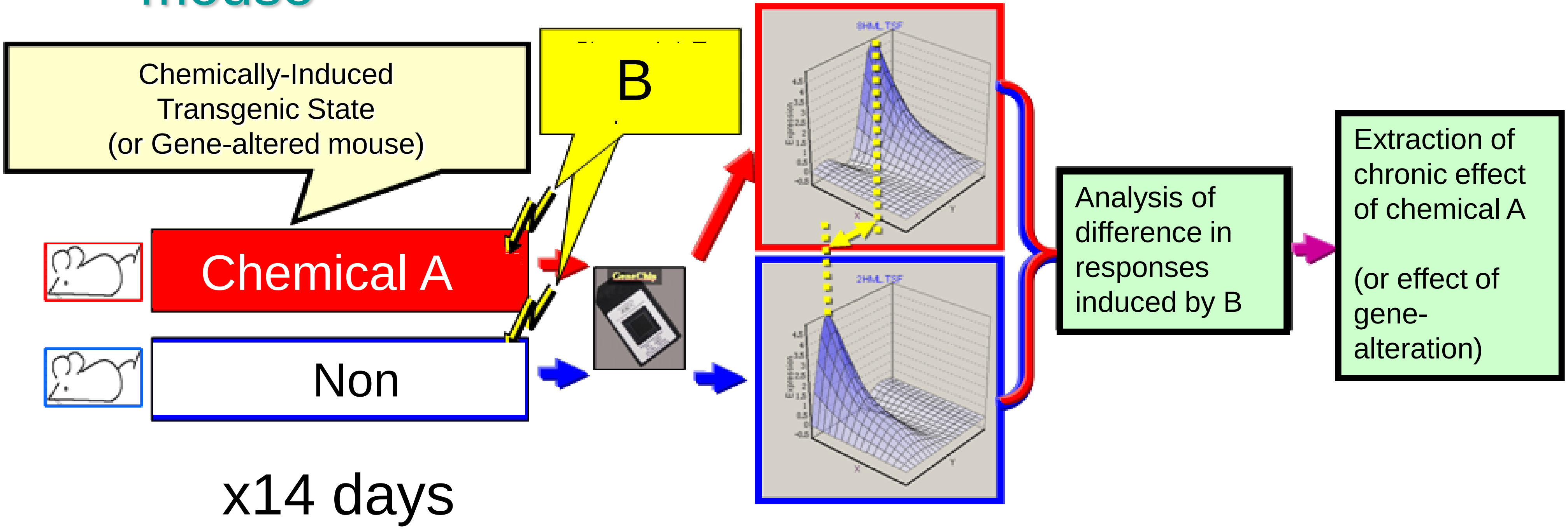
Extraction of chronic effect of chemical A

(or effect of gene-alteration)

Unpublished data

New Concept of Repeated Dose

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



Chemically-Induced
Transgenic State
(or Gene-altered mouse)

B

Chemical A

Non

x14 days

Analysis of difference in responses induced by B

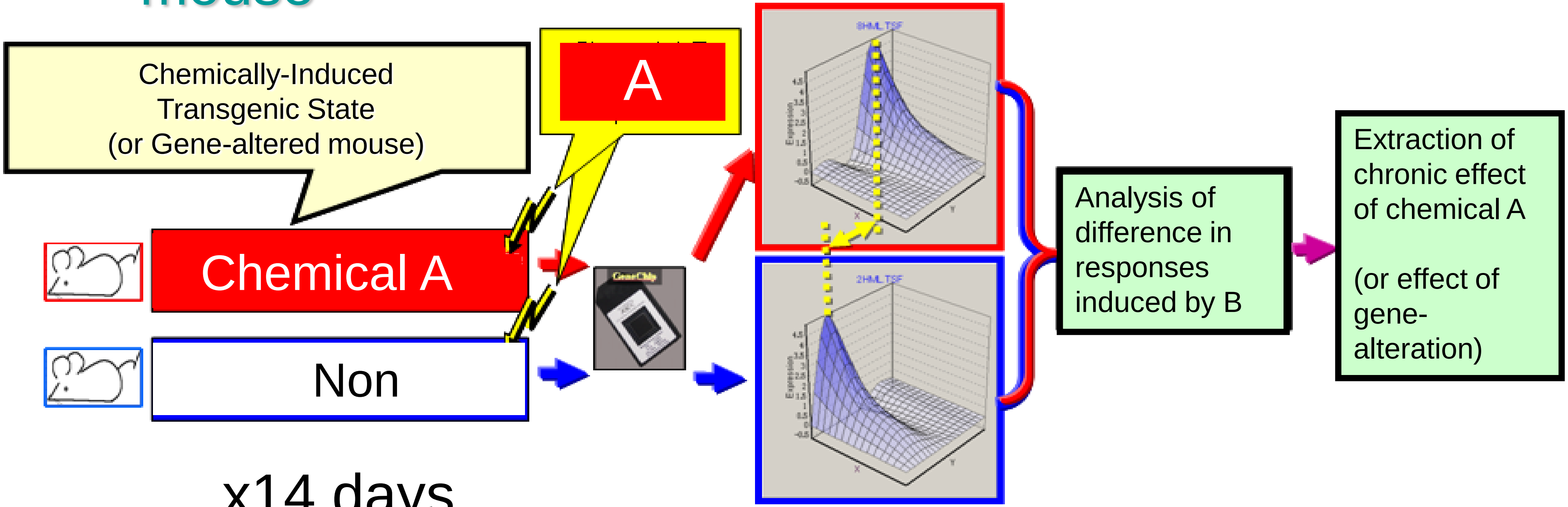
Extraction of chronic effect of chemical A

(or effect of gene-alteration)

Unpublished data

New Concept of Repeated Dose

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



Chemically-Induced
Transgenic State
(or Gene-altered mouse)

A

Chemical A

Non

x14 days

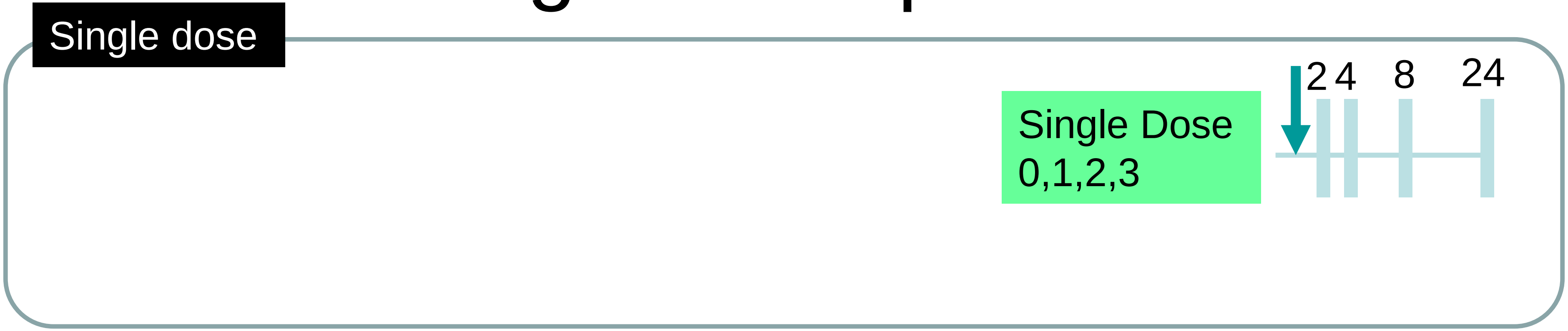
Analysis of difference in responses induced by B

Extraction of chronic effect of chemical A

(or effect of gene-alteration)

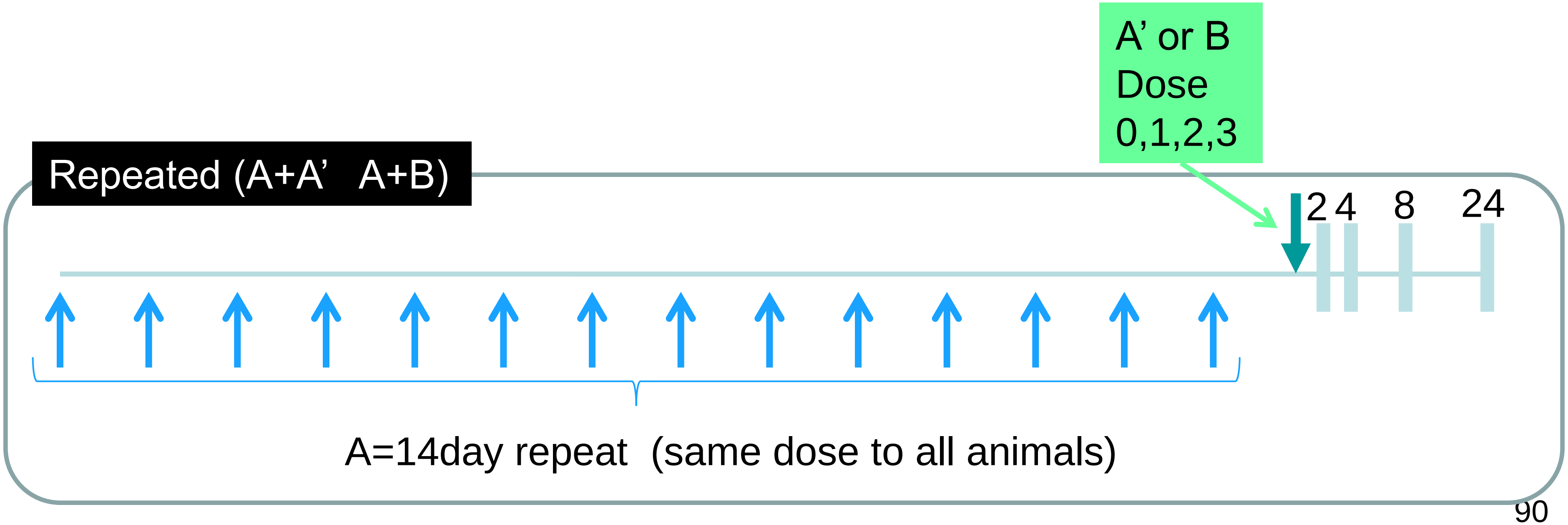
Unpublished data

Single vs Repeated



Single dose

Single Dose
0,1,2,3



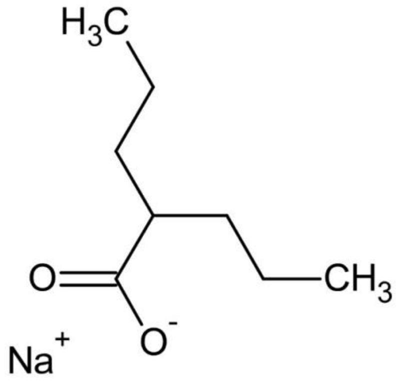
Repeated (A+A’ A+B)

A’ or B
Dose
0,1,2,3

A=14day repeat (same dose to all animals)

Unpublished data

Valproic acid sodium salt

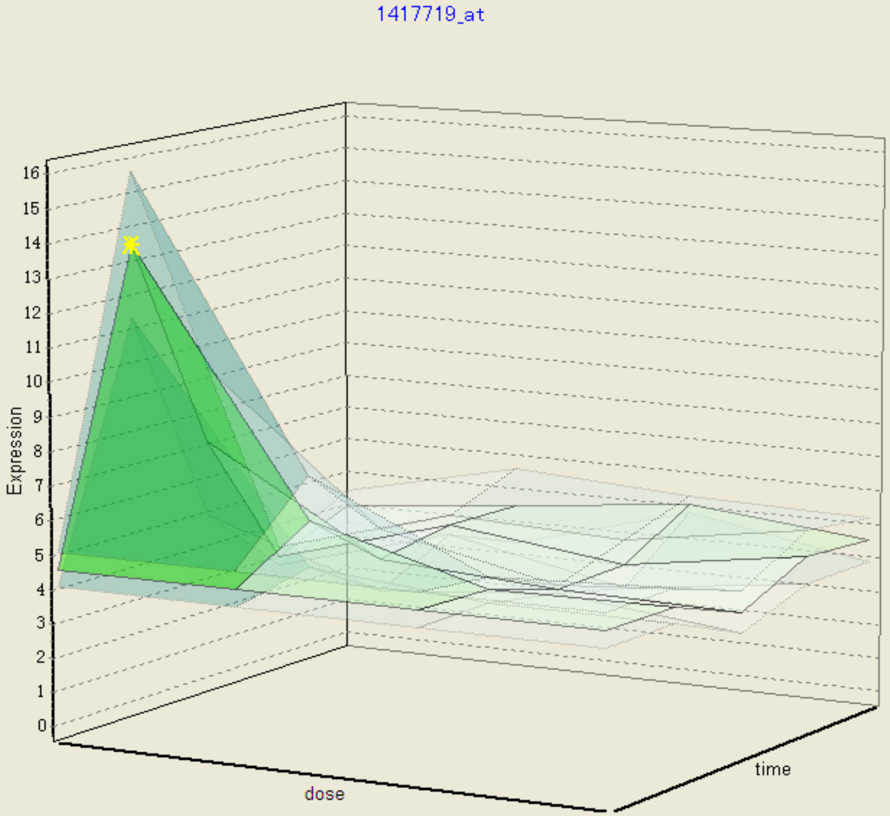


Single dose
[0, 50, 150, 500 mg/kg]

Repeated (A) and single (A’)
100 mg/kg (for 14 days) + [0, 50, 150, 500 mg/kg]

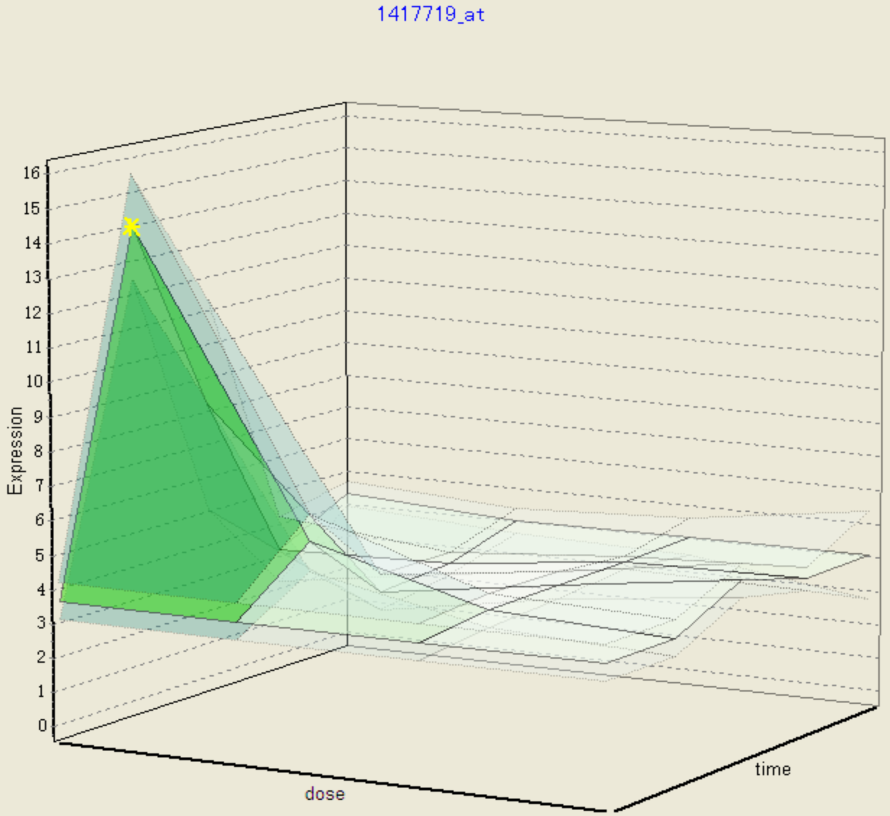
Unpublished data

TTG157-L_SpNC_0



VPA single

TTG151-L_SpNC_0

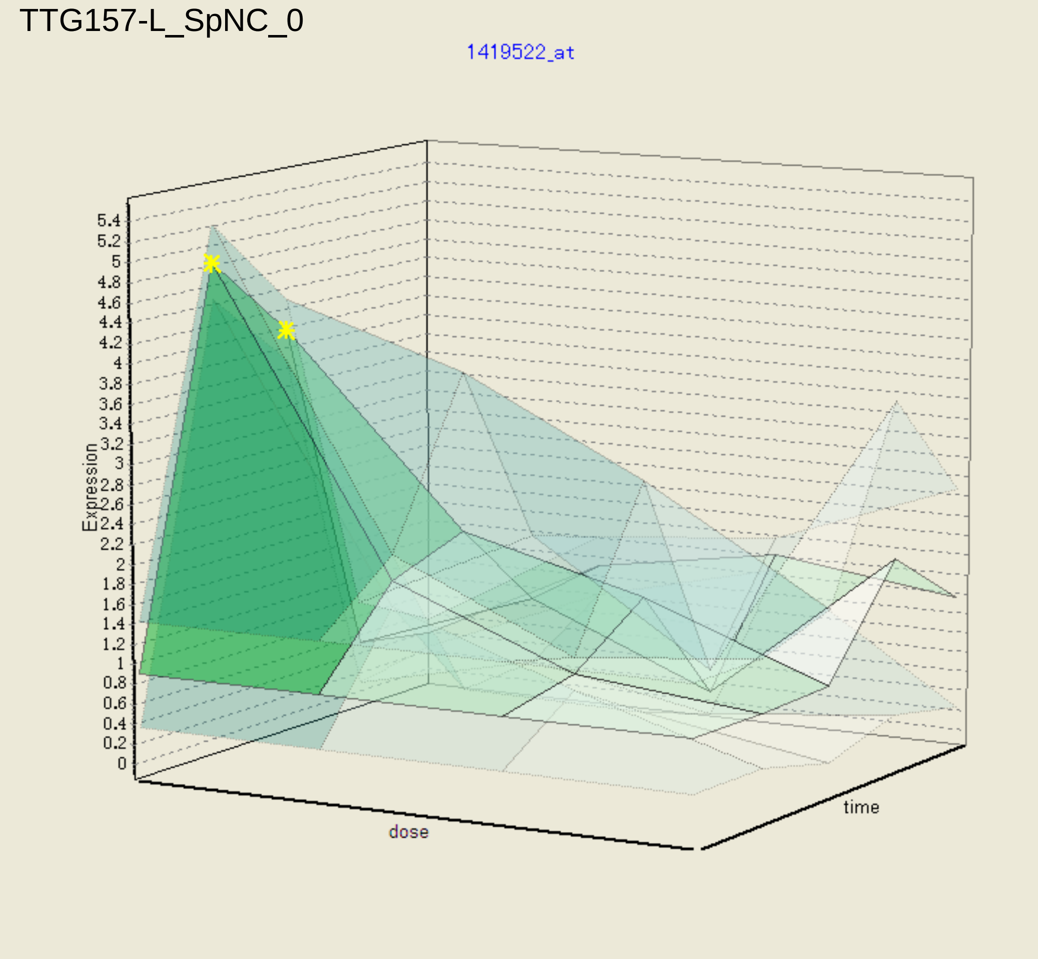


VPA repeat

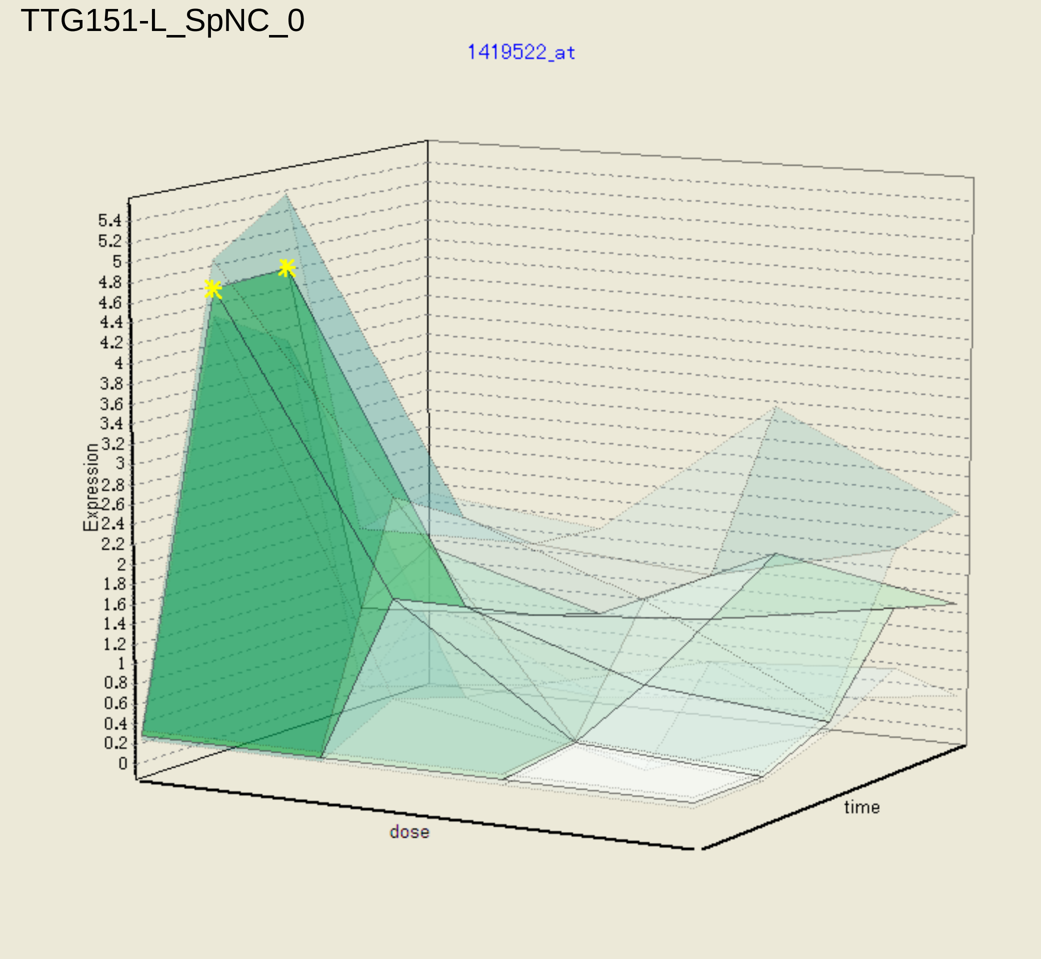
Sap30
sin3 associated polypeptide

92

Unpublished data



VPA single

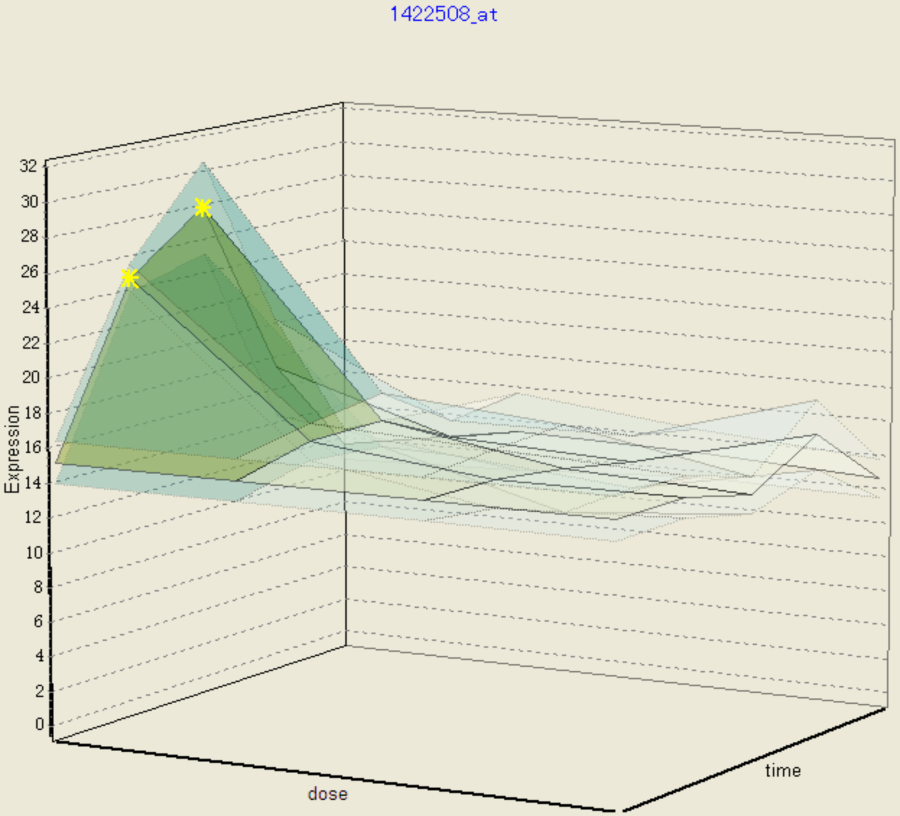


VPA repeat

Zmynd19
zinc finger, MYND domain containing 19

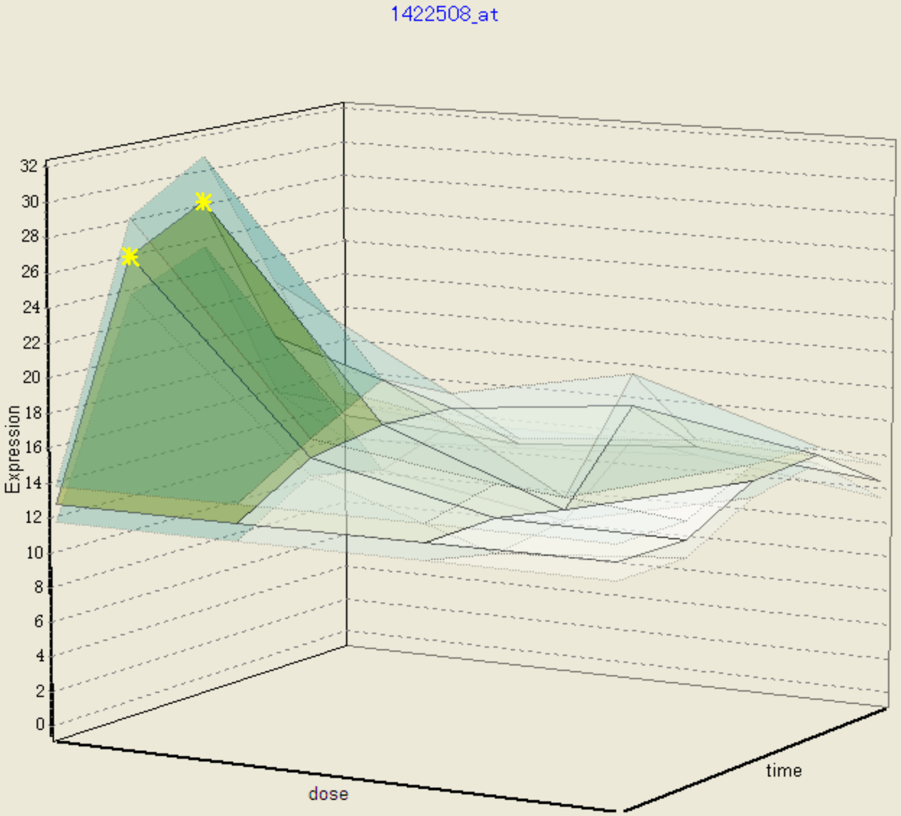
Unpublished data

TTG157-L_SpNC_0



VPA single

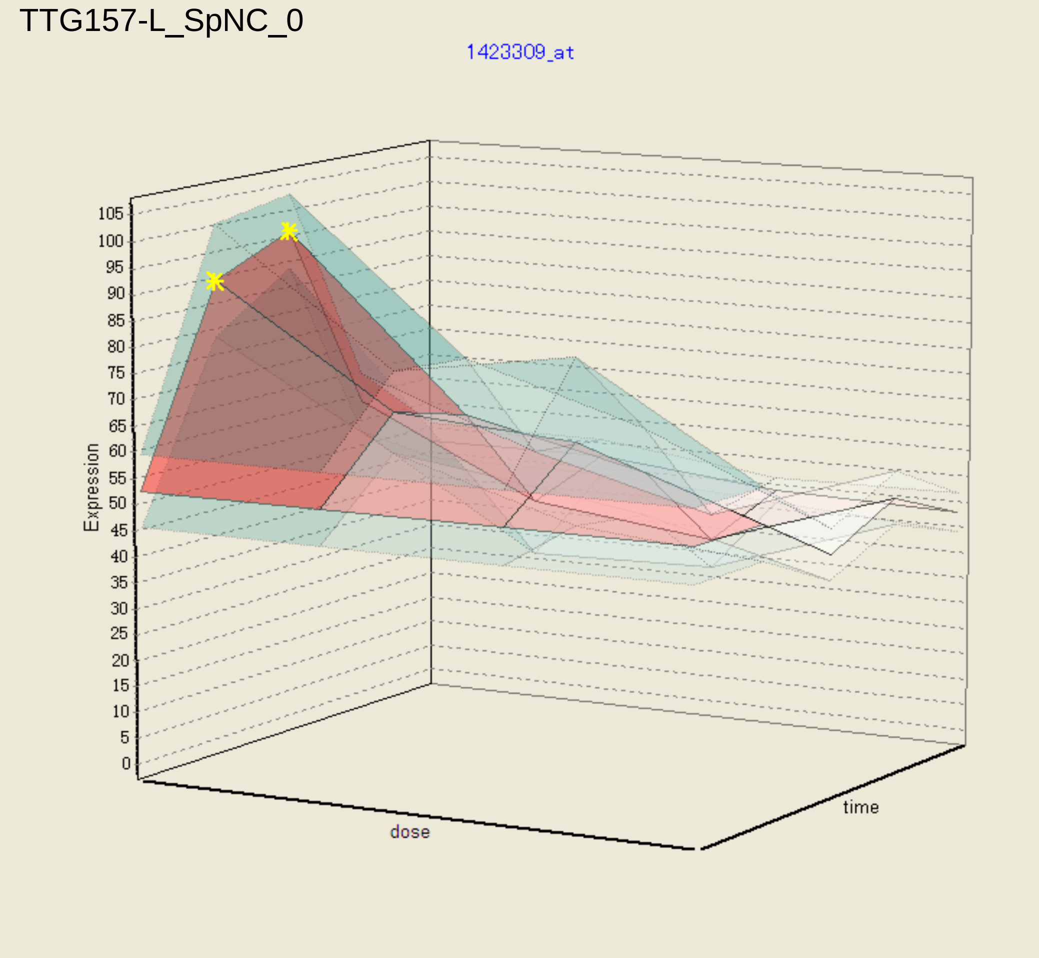
TTG151-L_SpNC_0



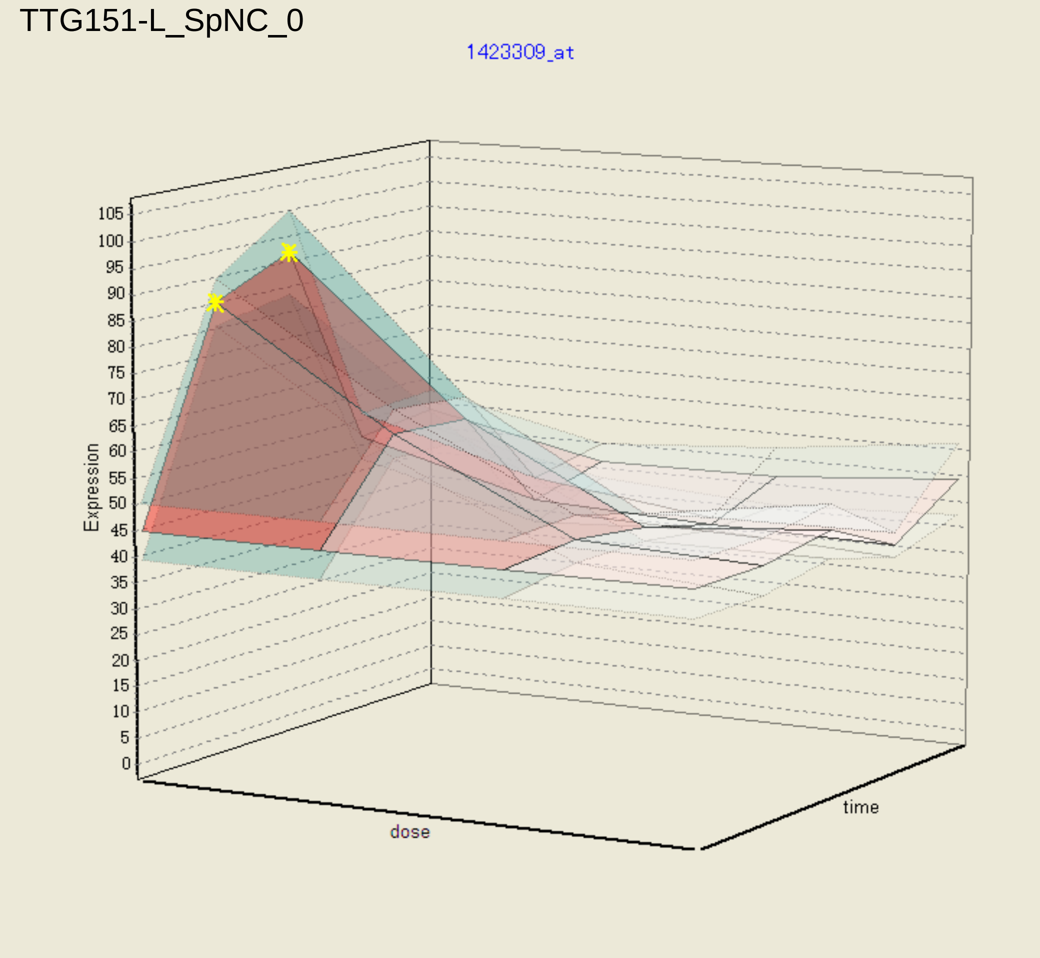
VPA repeat

Atp6v1a
ATPase, H+ transporting, lysosomal V1 subunit A

Unpublished data



VPA single

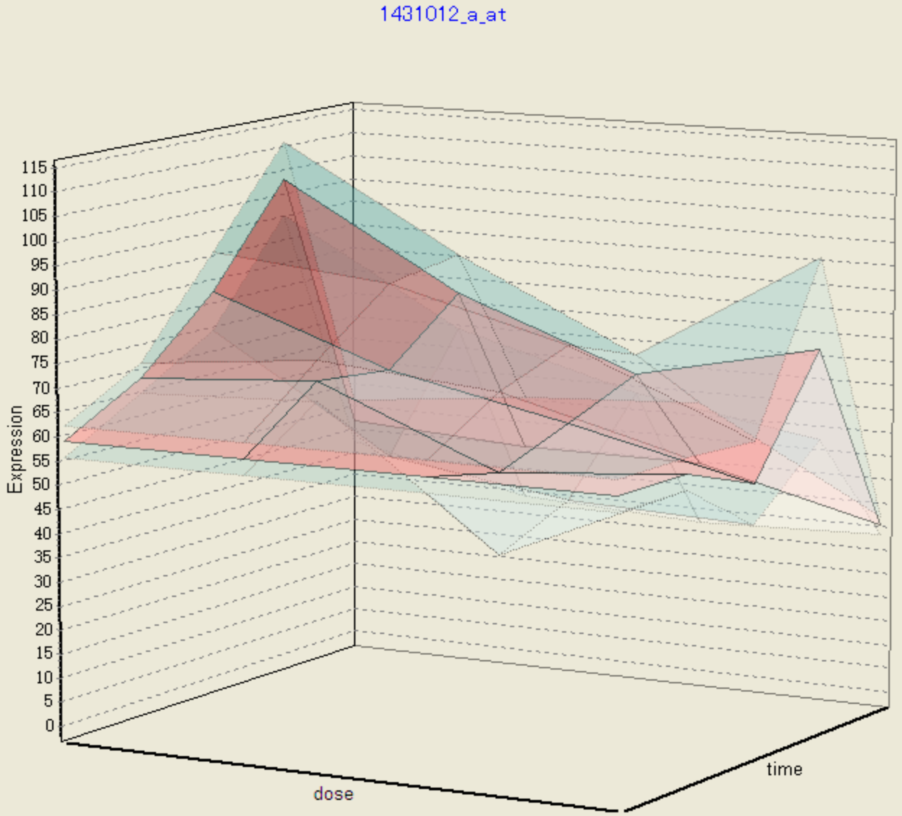


VPA repeat

Tgoln1 /// Tgoln2
trans-golgi network protein /// trans-golgi network protein 2

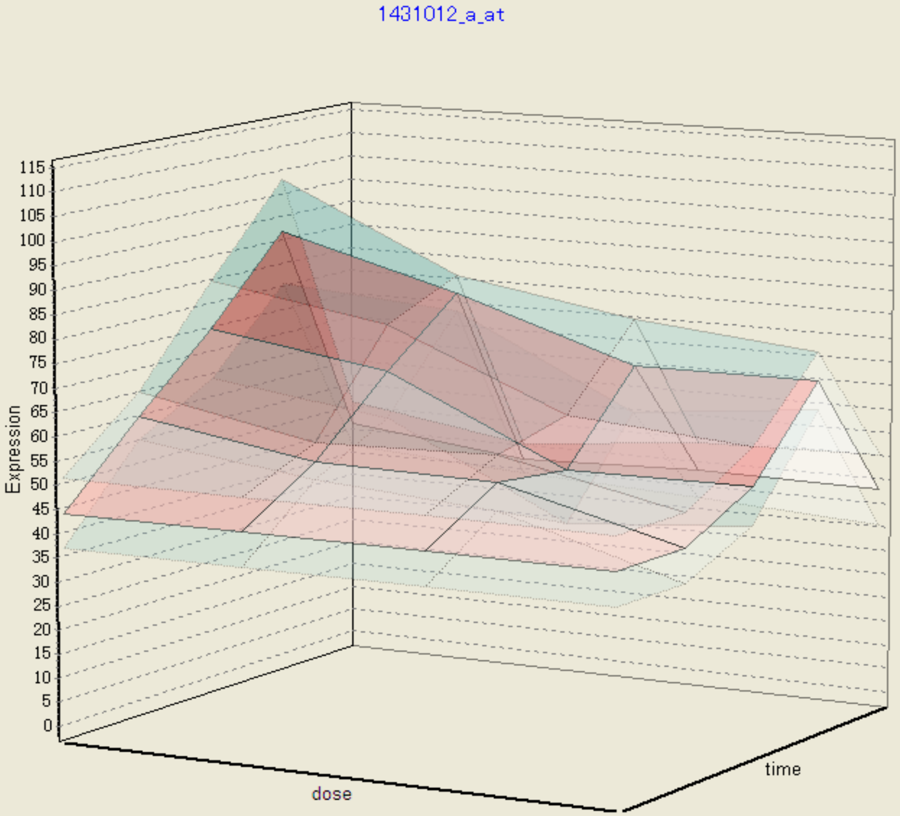
Unpublished data

TTG157-L_SpNC_0



VPA single

TTG151-L_SpNC_0

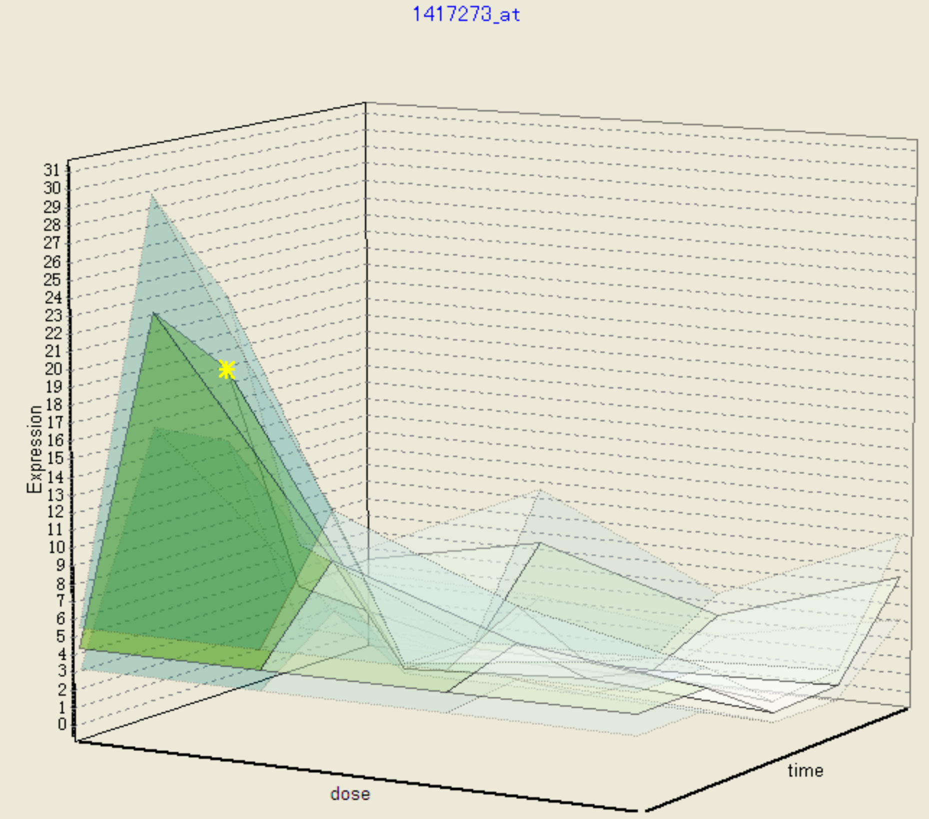


VPA repeat

Peci
peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase

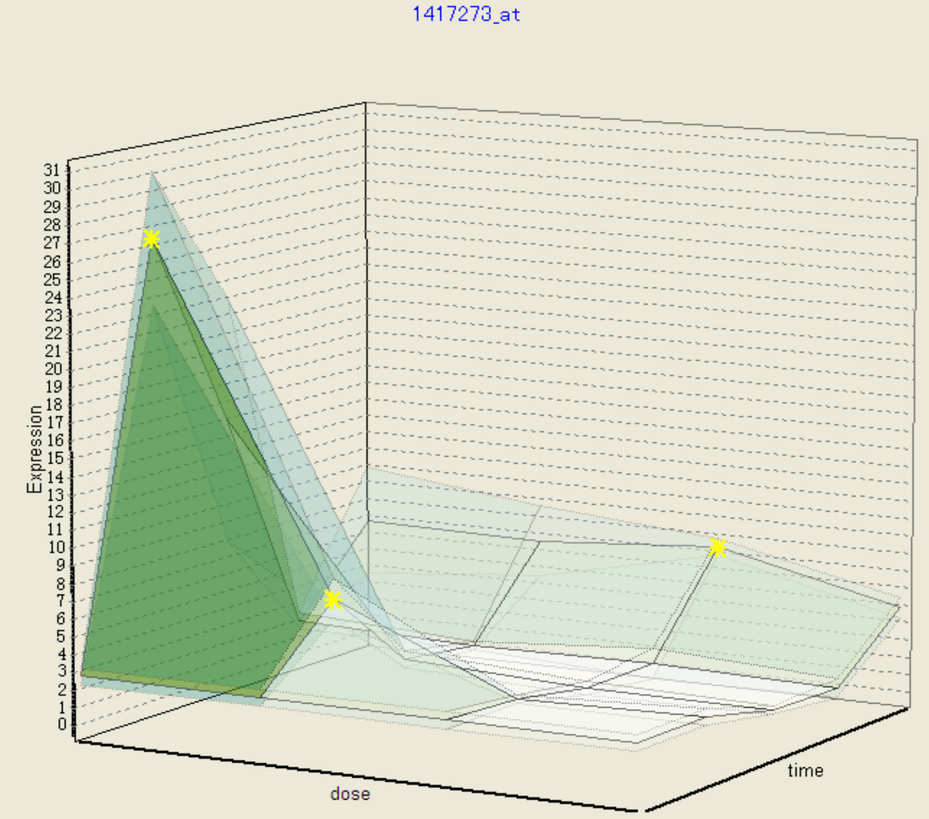
Unpublished data

TTG157-L_SpNC_0



VPA single

TTG151-L_SpNC_0

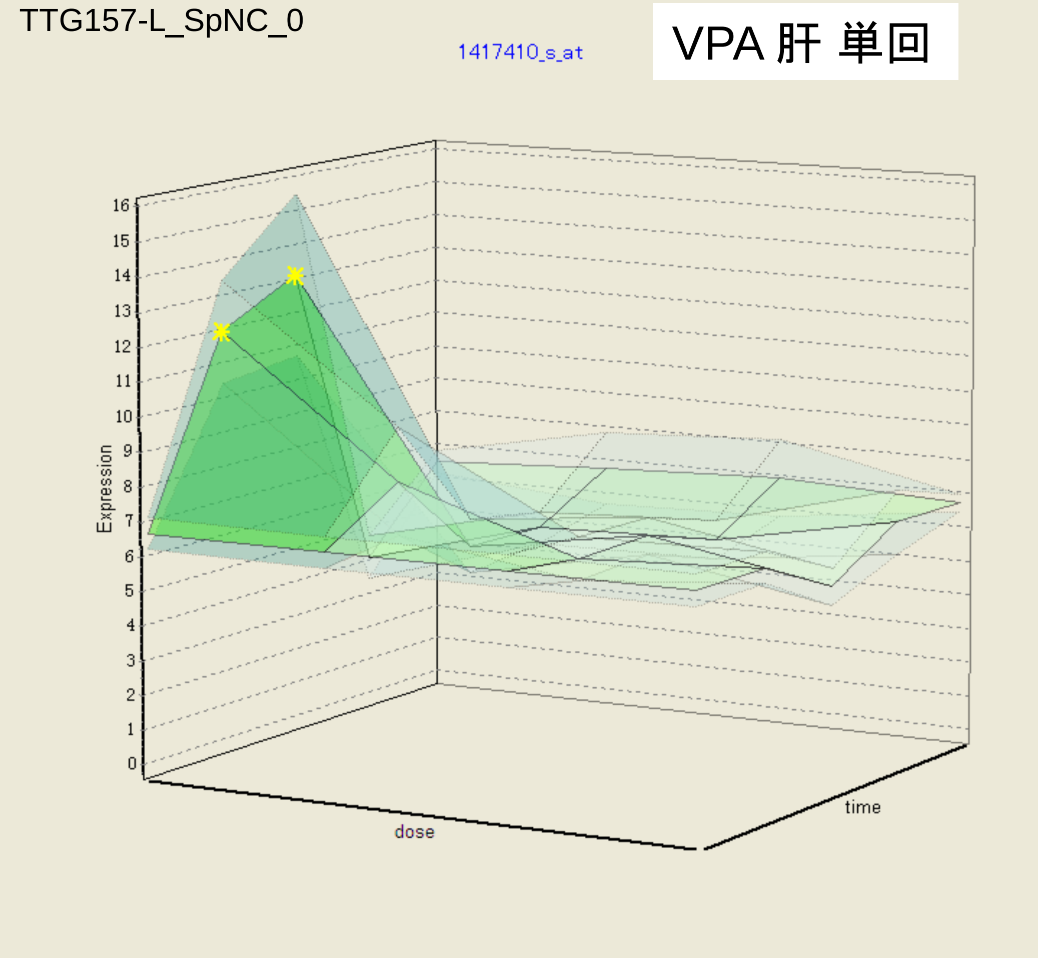


VPA repeat

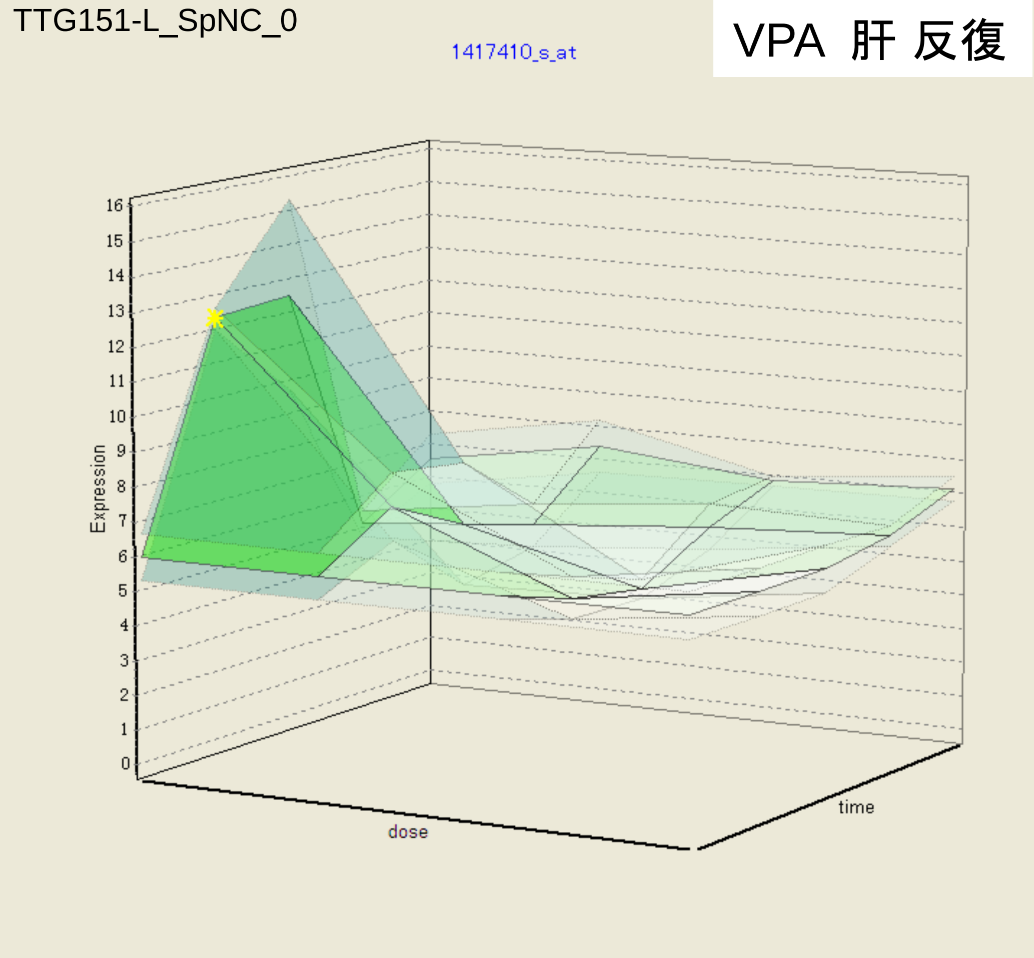
Pdk4
pyruvate dehydrogenase kinase, isoenzyme 4

97

Unpublished data



VPA single



VPA repeat

Prkci
protein kinase C, iota

Unpublished data

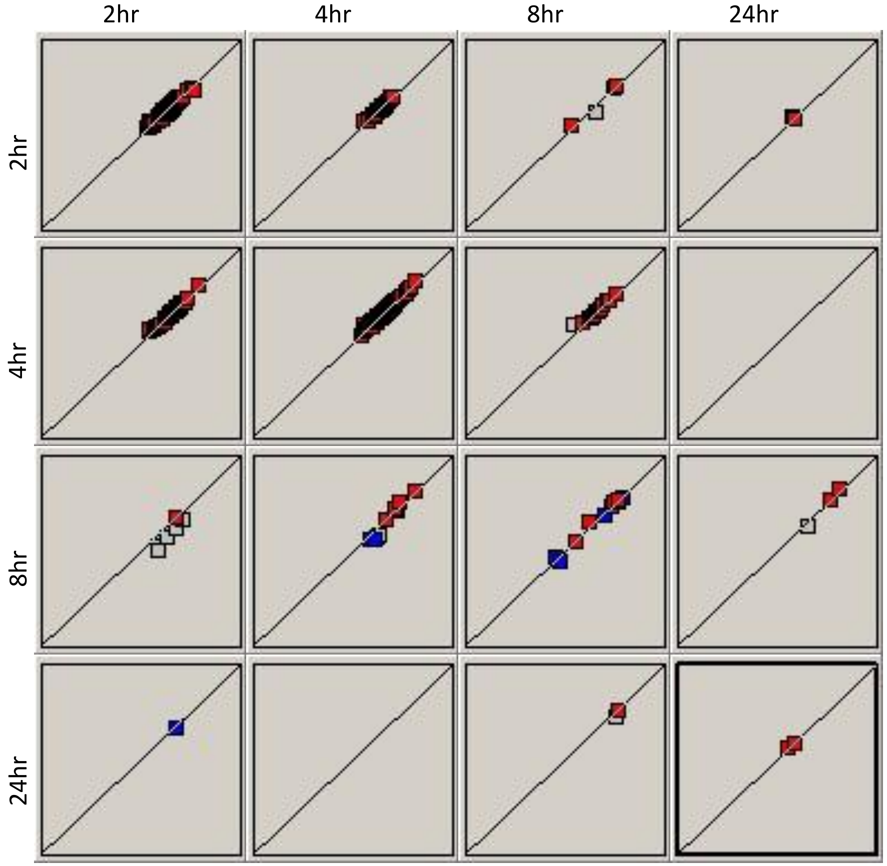
Percellome Explorer解析

Valproic acid sodium salt

- PDBEx_RSort101008_Std-Med.PDBEx
- Target*Candidate

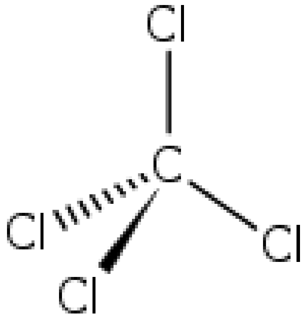
Repeat A+A’

Single



Unpublished data

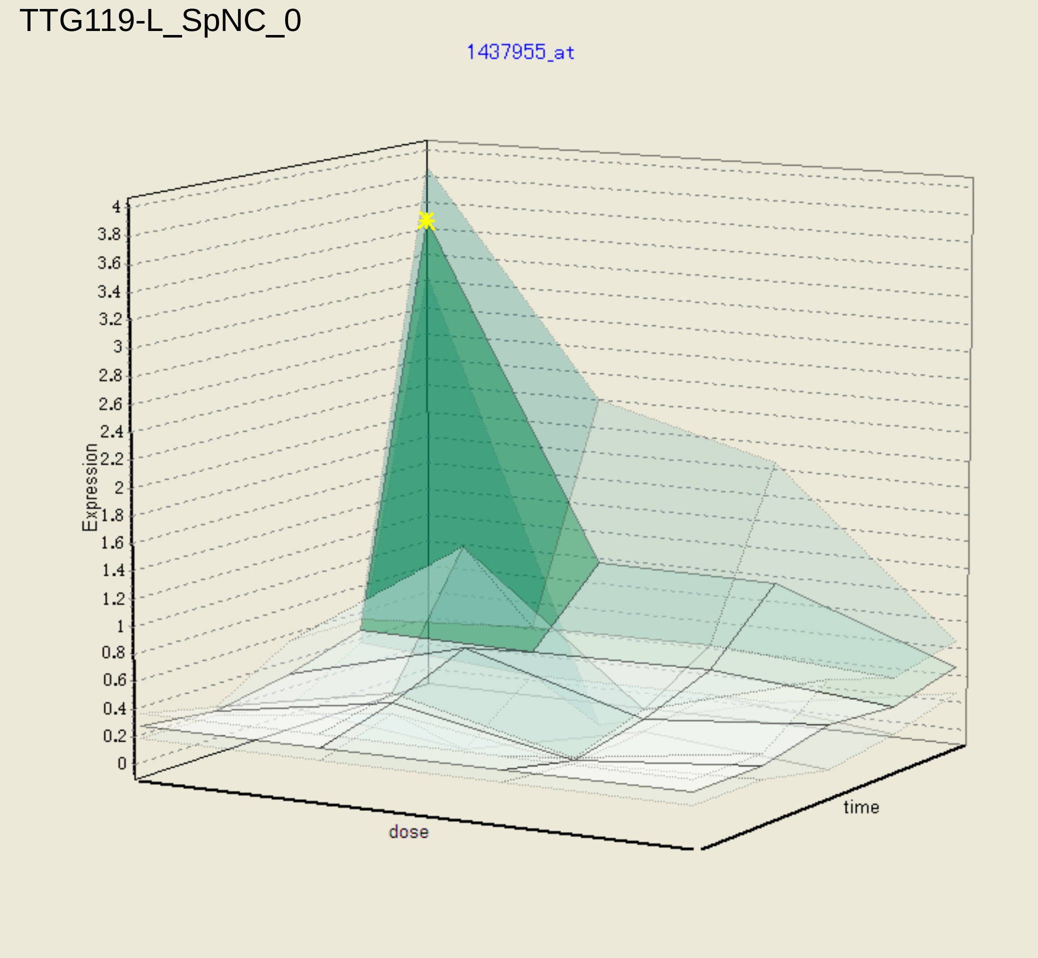
Carbon tetrachloride



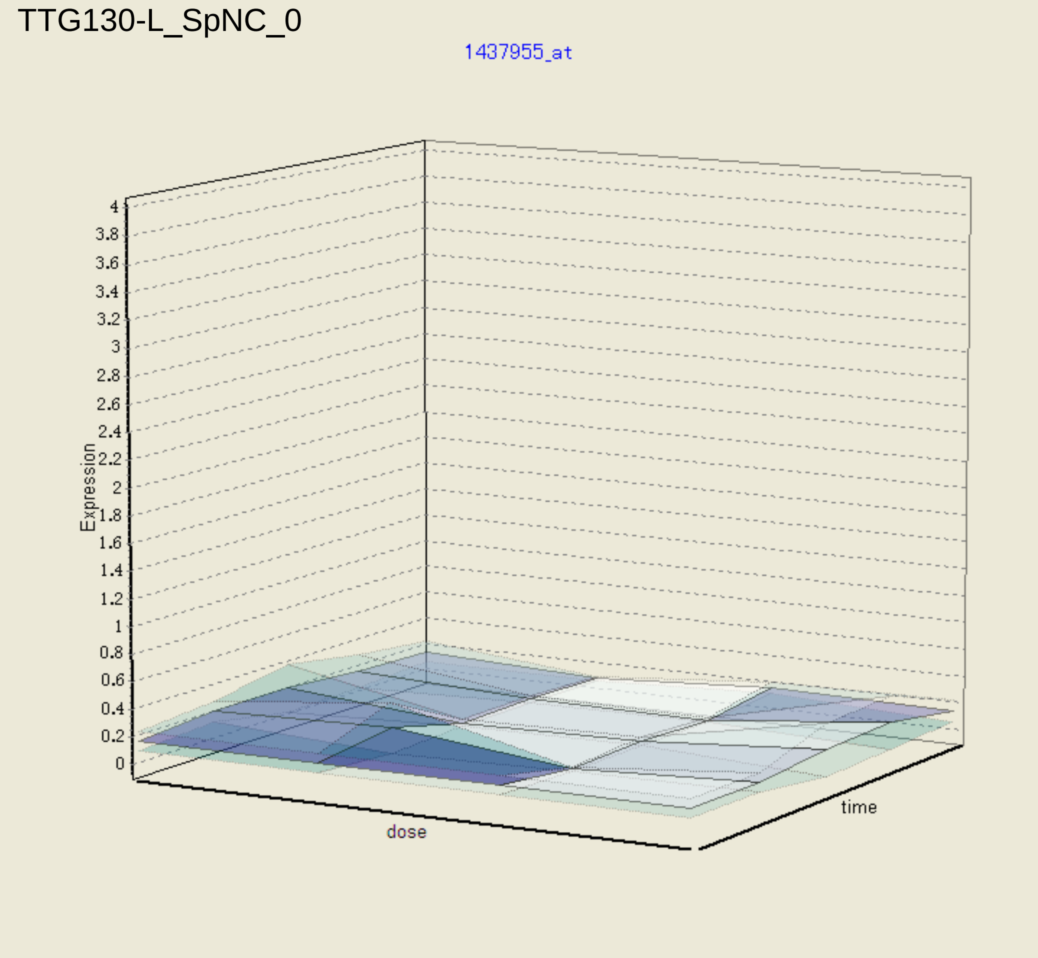
Single dosing
[0, 0.7, 2, 7 mg/kg]

Repeat + Single
5 mg/kg (for 14 days to all animals) +   [0, 0.7, 2, 7 mg/kg]

Unpublished data



CCl$_4$ single



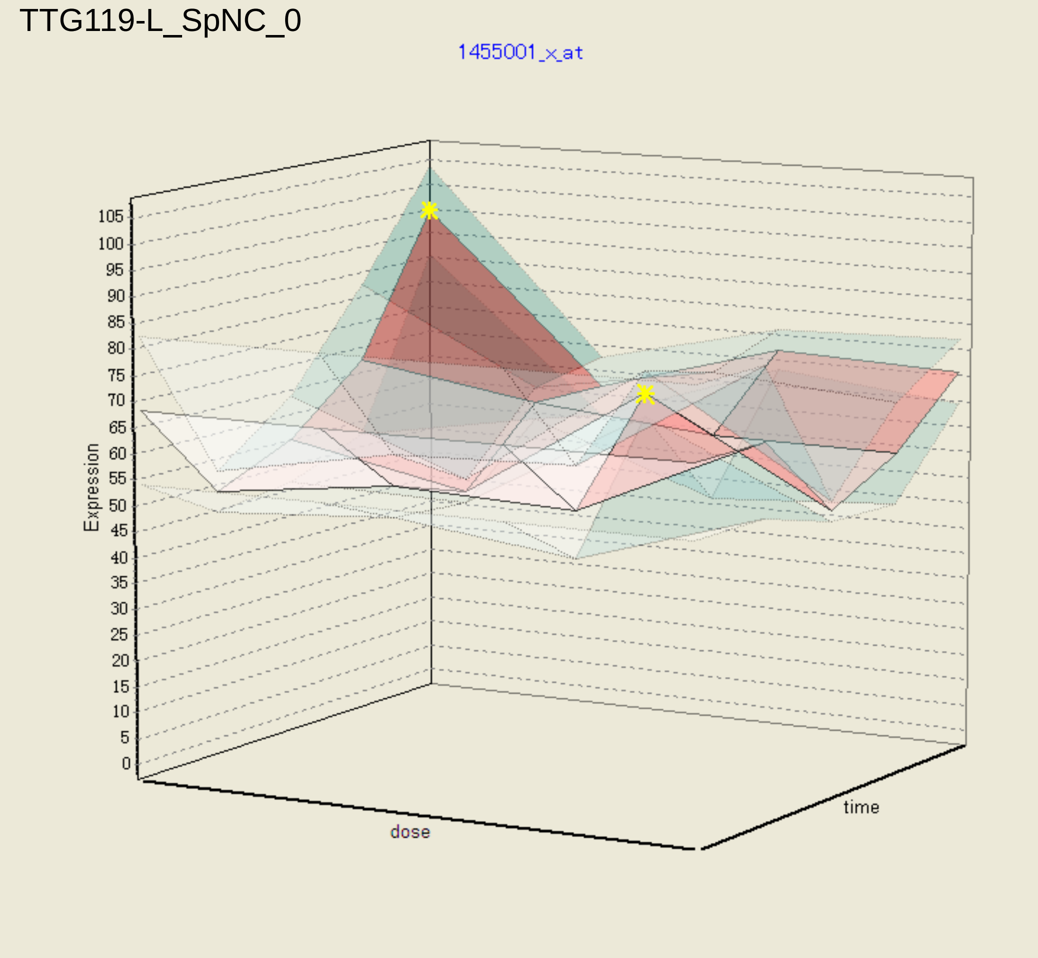
CCl$_4$ repeat

Gene showing Attenuated response

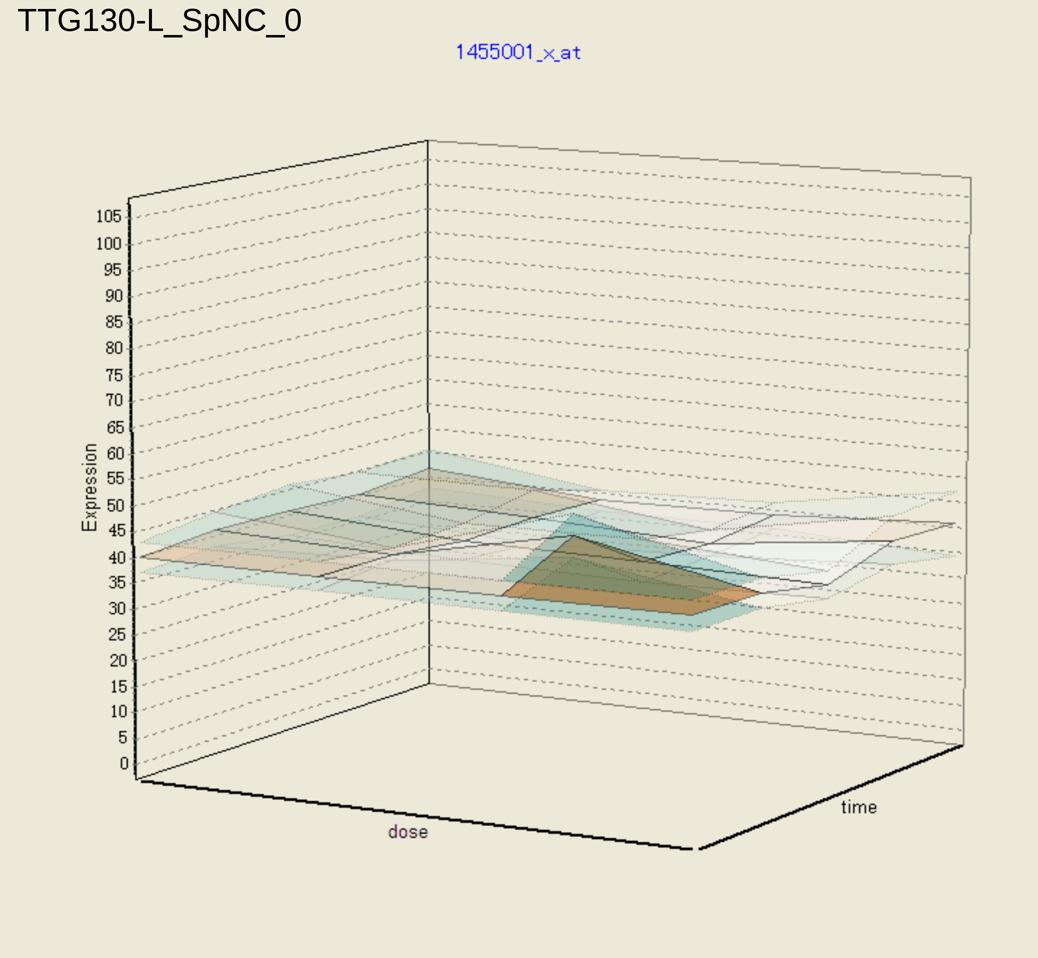
Ubqlnl
ubiquilin-like

101

Unpublished data



CCl_4 single



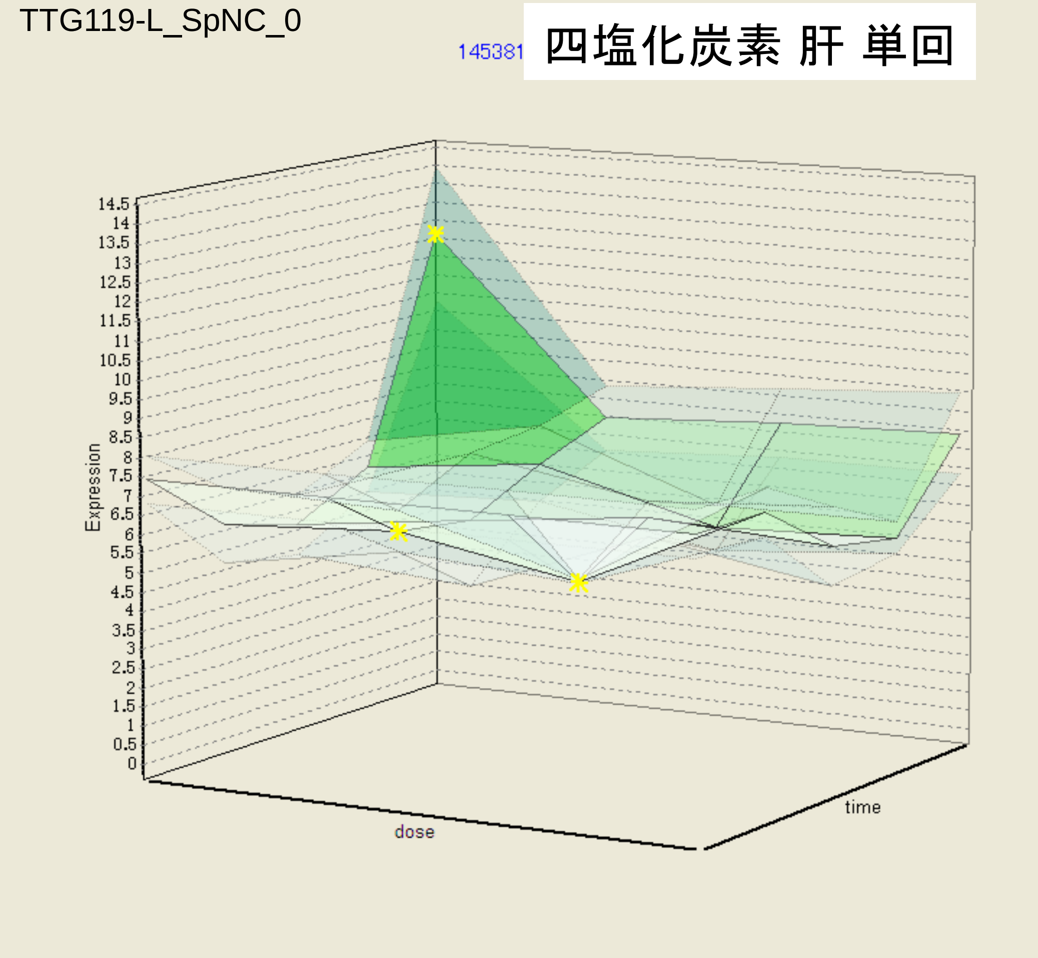
CCl_4 repeat

Gene showing Attenuated response

Rpl13a
ribosomal protein L13a

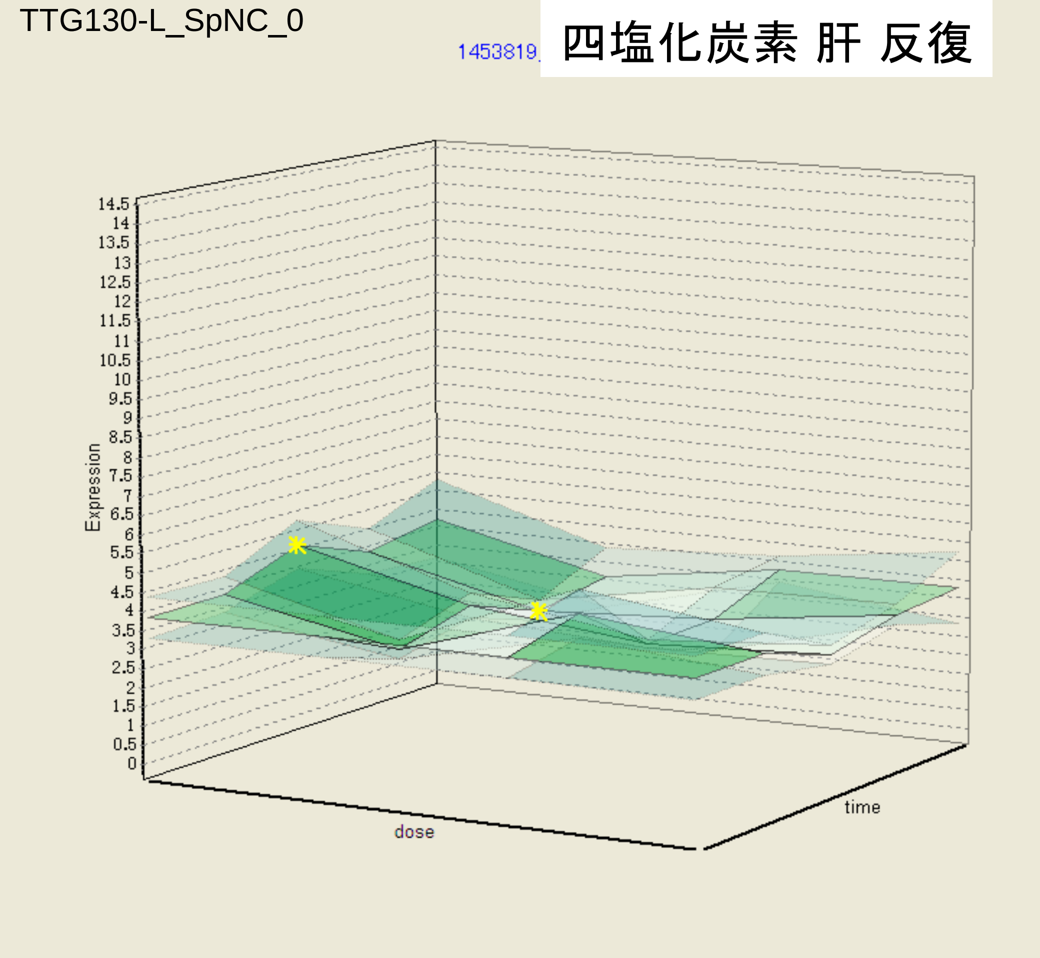
102

Unpublished data



四塩化炭素 肝 単回

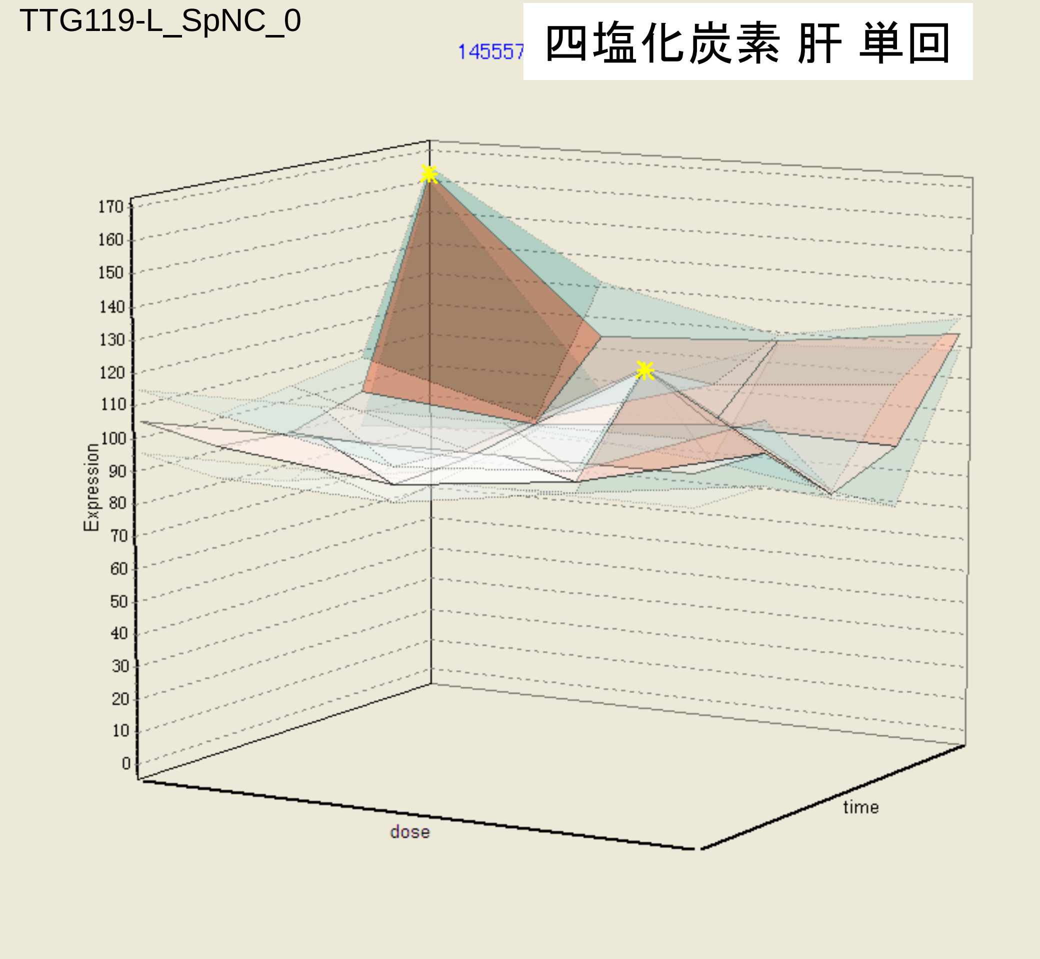
CCl$_4$ single



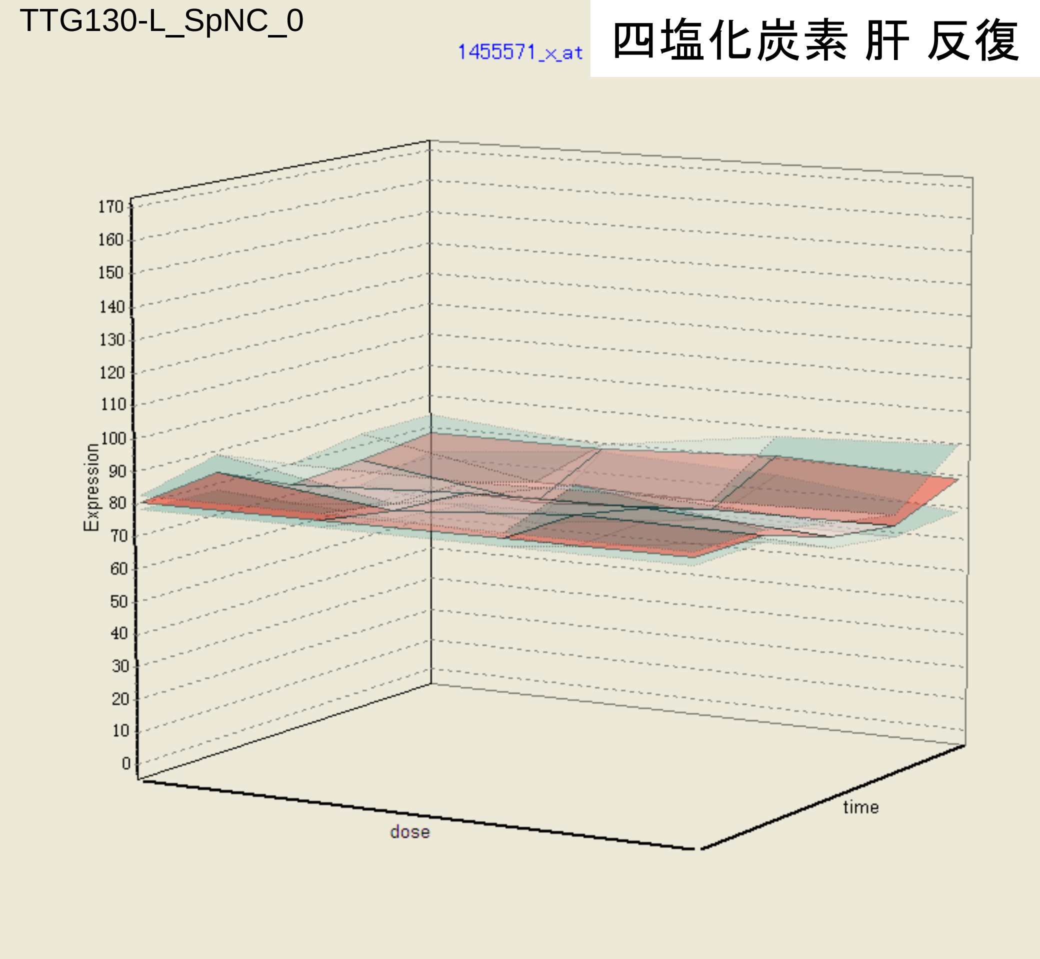
四塩化炭素 肝 反復

CCl$_4$ repeat

Stx18
syntaxin 18

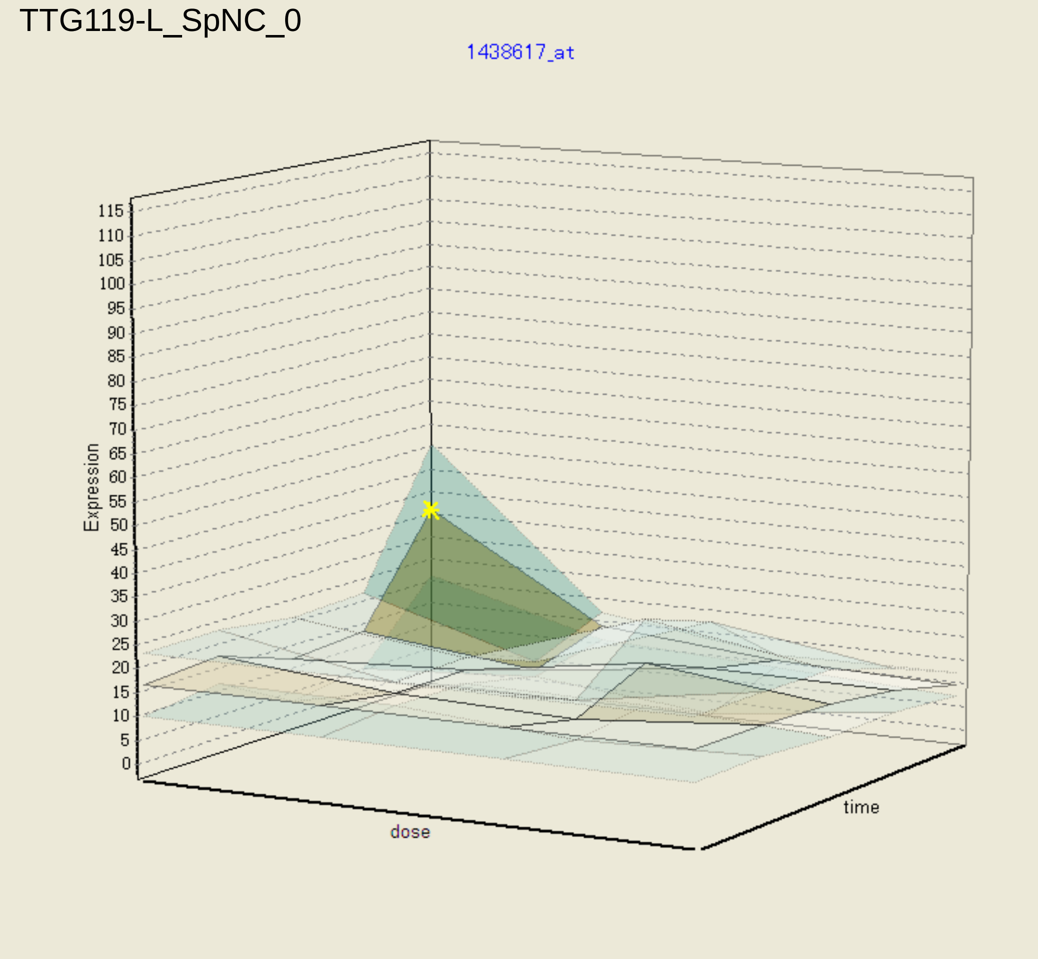


CCl$_4$ single

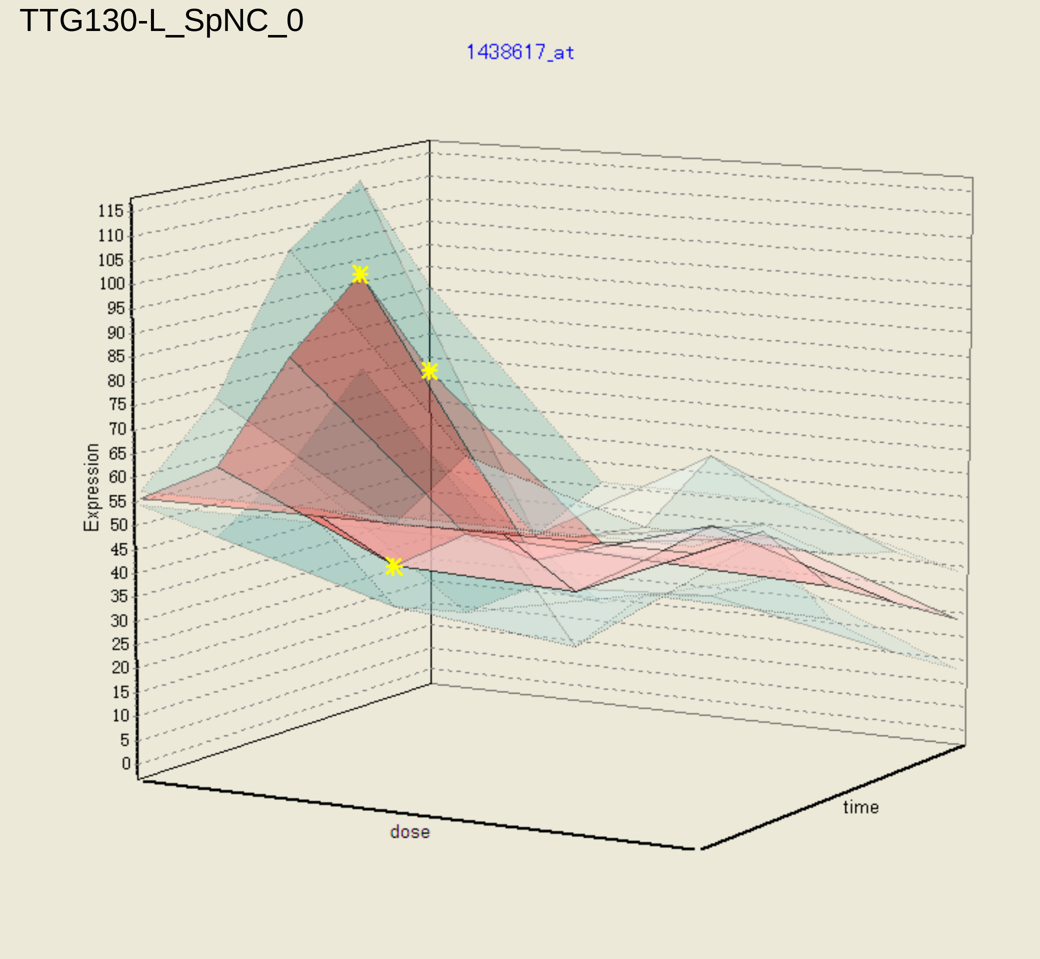


CCl$_4$ repeat

Calm1
calmodulin 1



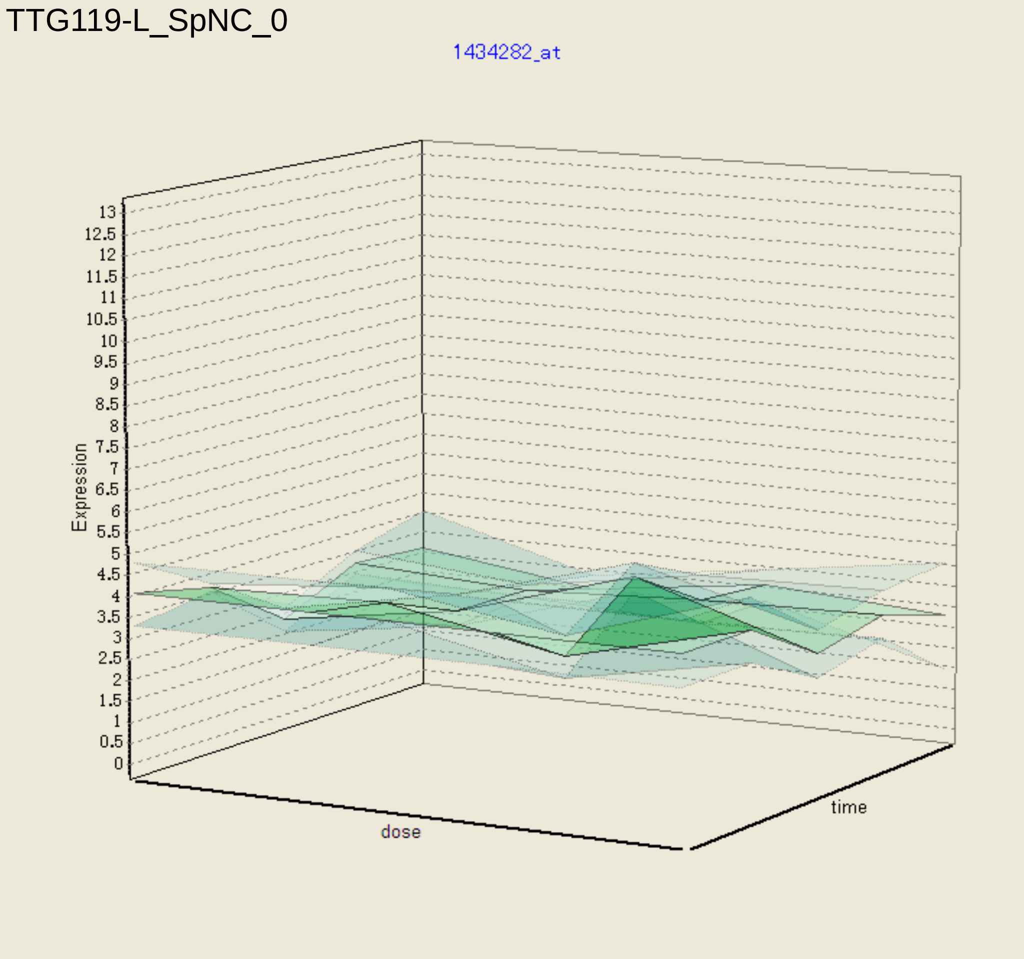
CCl$_4$ single



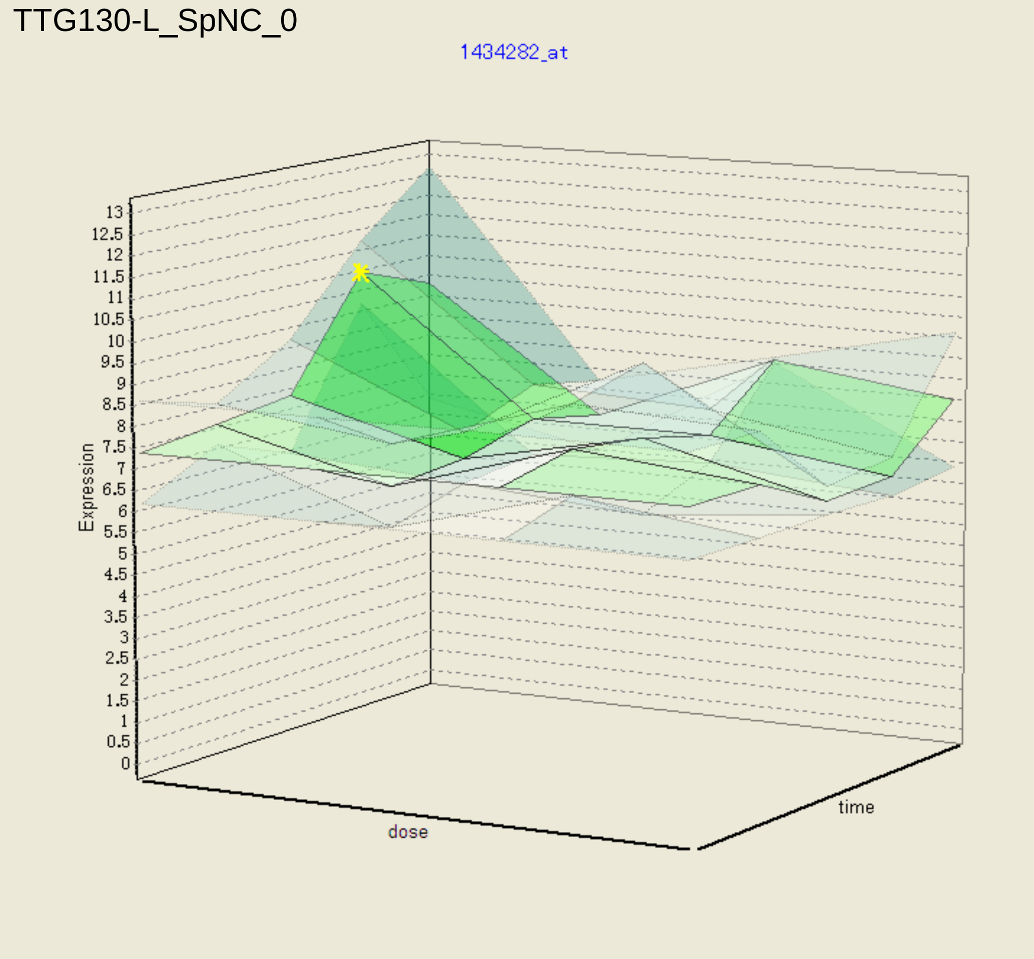
CCl$_4$ repeat

Gene showing Enhanced response

Serpina7
serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7



CCl$_4$ single

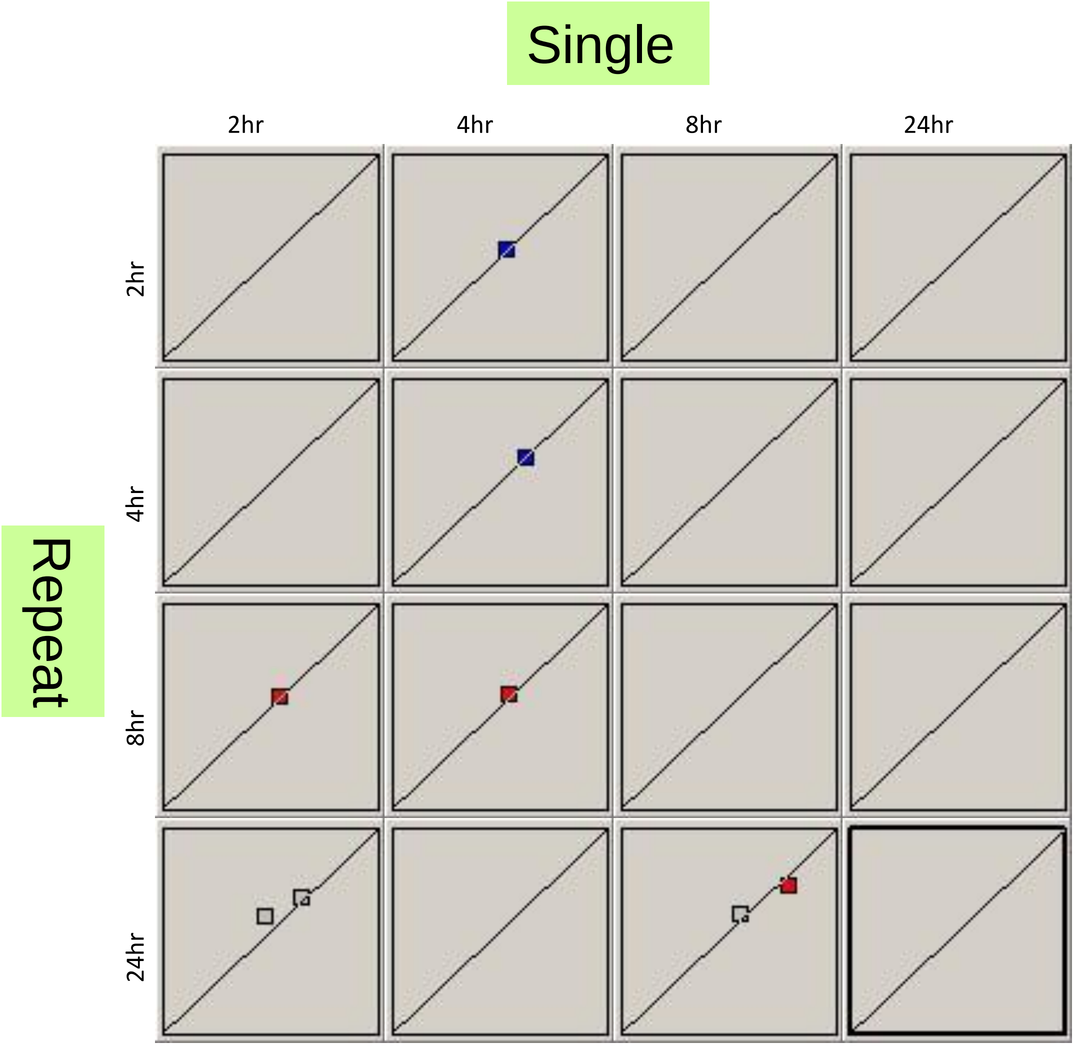


CCl$_4$ repeat

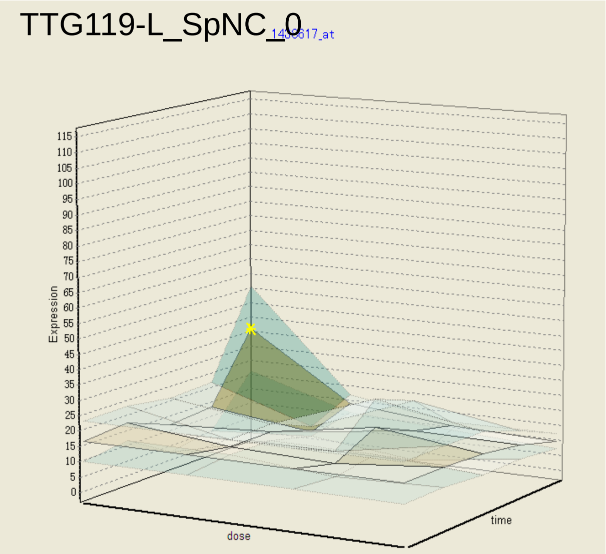
Gene showing Enhanced response

Ibtk
inhibitor of Bruton agammaglobulinemia tyrosine kinase

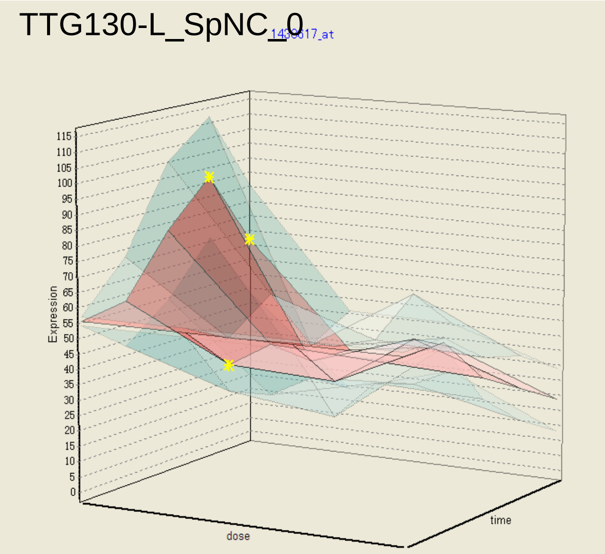
Carbon tetrachloride



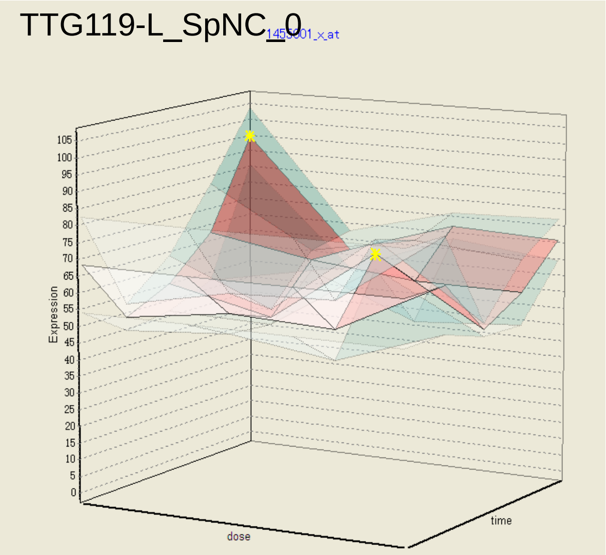
Possible General Rule !



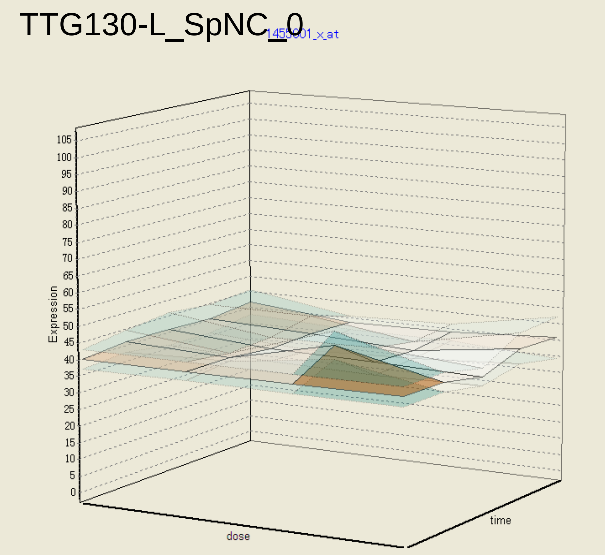
Single



Repeated



Single



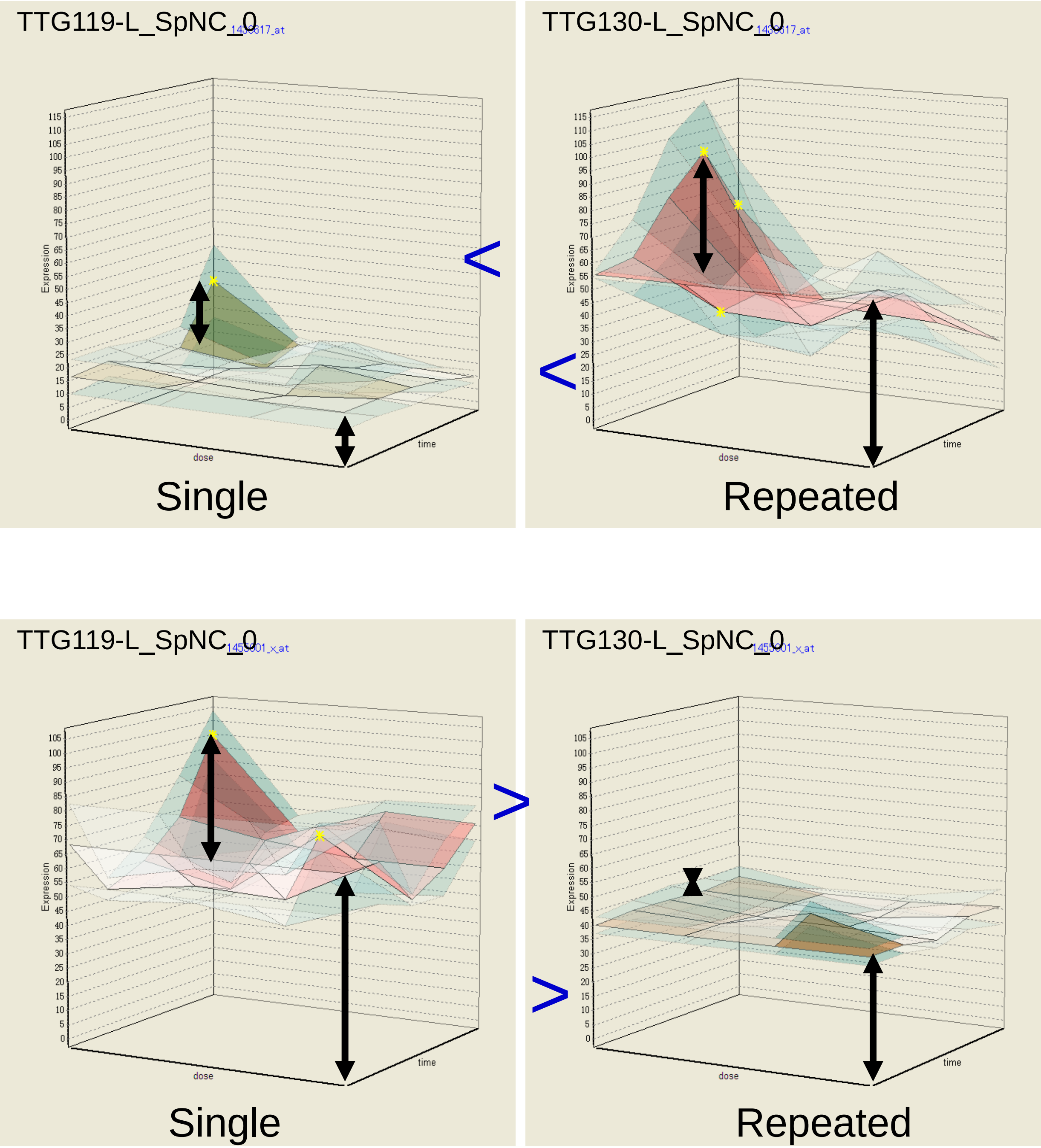
Repeated

Unpublished data

Possible General Rule

Induction is in relation to Base-line copy number!

Q: what signal induces this base-line changes?



Interpretation of the finding

A+A’ protocol experiment suggested that

Chemicals can be classified as either

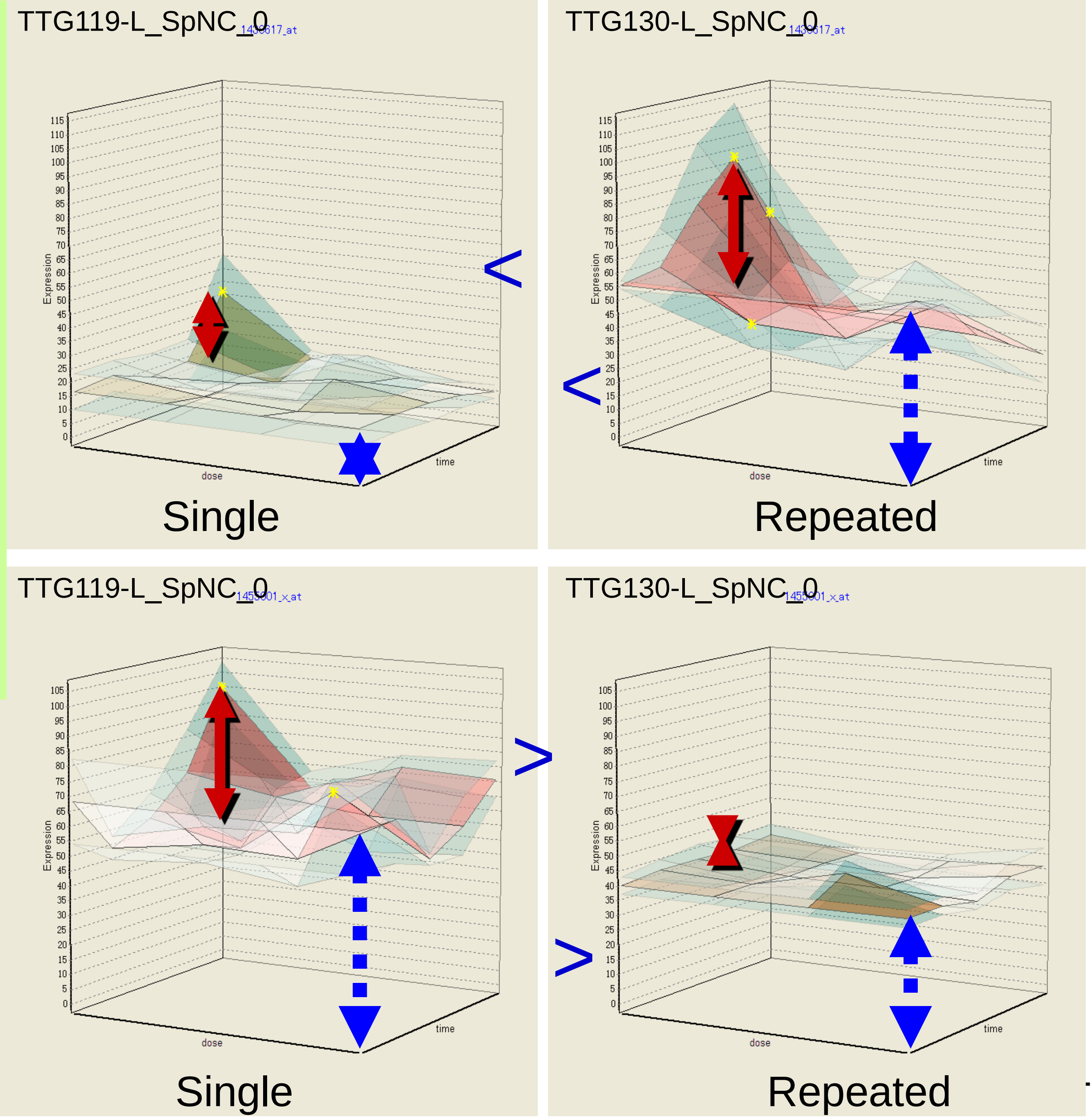
●Repeated dosing does NOT affect the response of Single
or
■Repeated dosing DOES affect the response of Single

■Repeated dosing DOES affect the response of Single

Definition用語定義

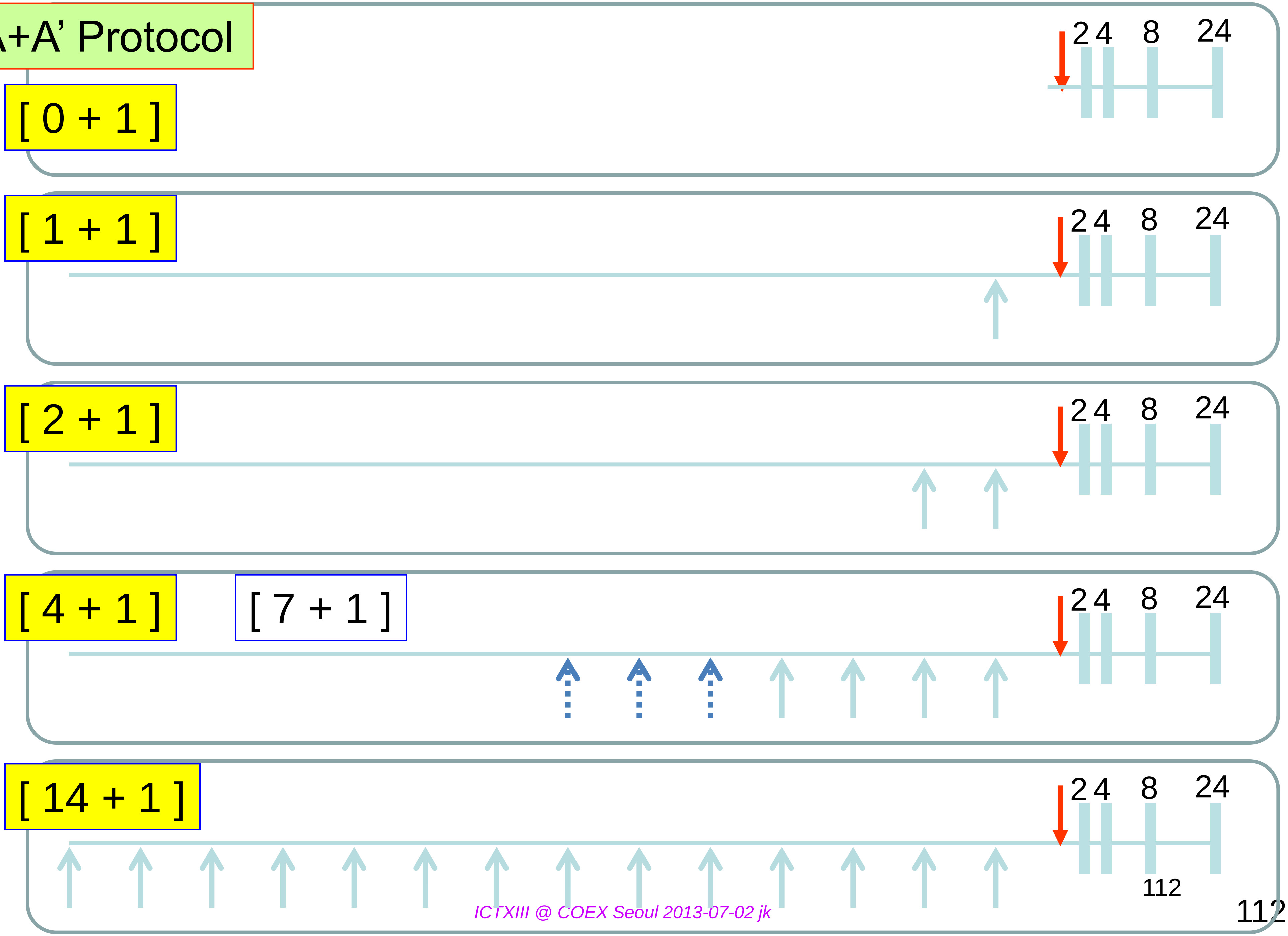
↕ Transient Response
(T-Res)
過渡反応

⇕ Baseline Response
(B-Res)
基線反応



Unpublished data

A+A’ Protocol


[0 + 1]
2 4 8 24
[1 + 1]
2 4 8 24
[2 + 1]
2 4 8 24
[4 + 1]
[7 + 1]
2 4 8 24
[14 + 1]
2 4 8 24

遺伝子発現変動
3Dグラフ
配置図

[0 + 1]

単回暴露
(TTG119)

[2 + 1]

2日間反復暴露
(TTG190)

[4 + 1]

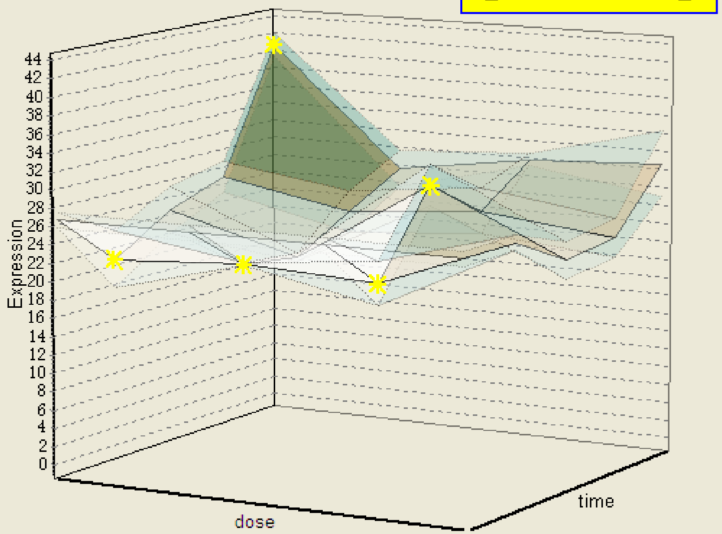
4日間反復暴露
(TTG191)

[14 + 1]

14日間反復暴露
(TTG130)

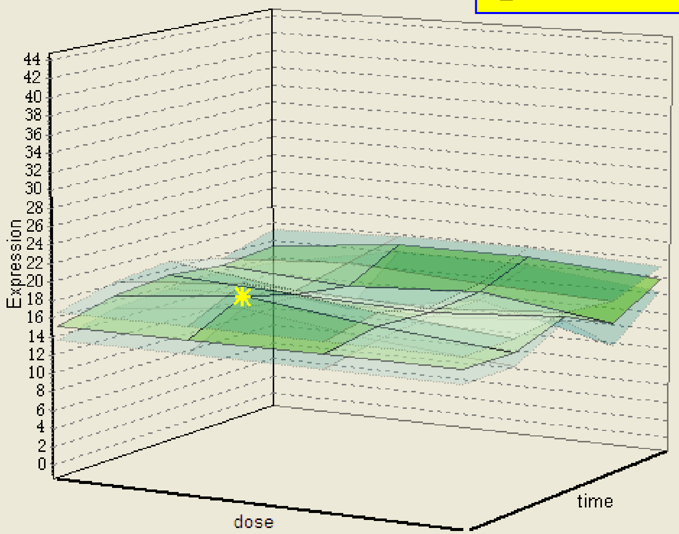
TTG119-L_SpNC_0 1415762_x_at

[0 + 1]



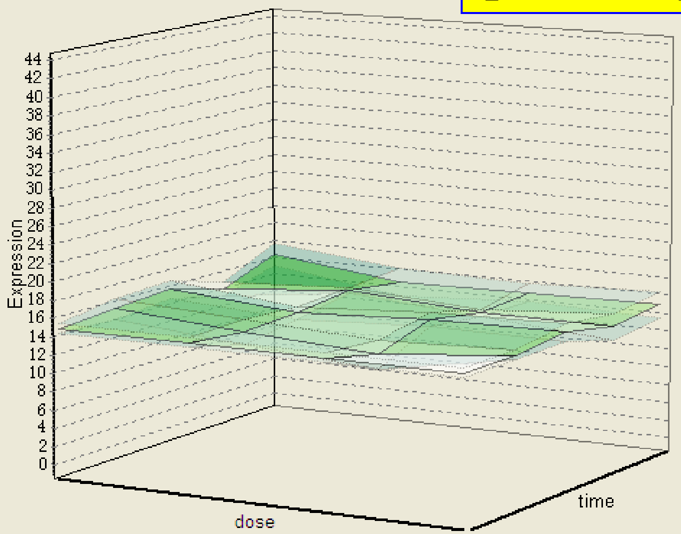
TTG190-L_SpNC_0 1415762_x_at

[2 + 1]



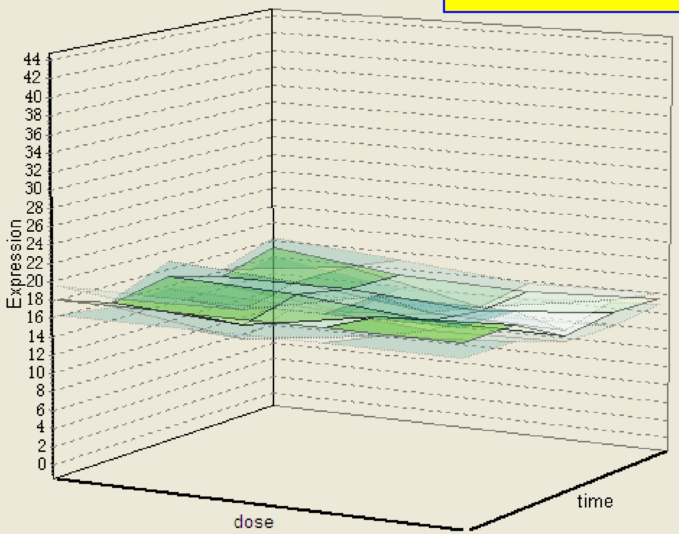
TTG191-L_SpNC_0 1415762_x_at

[4 + 1]



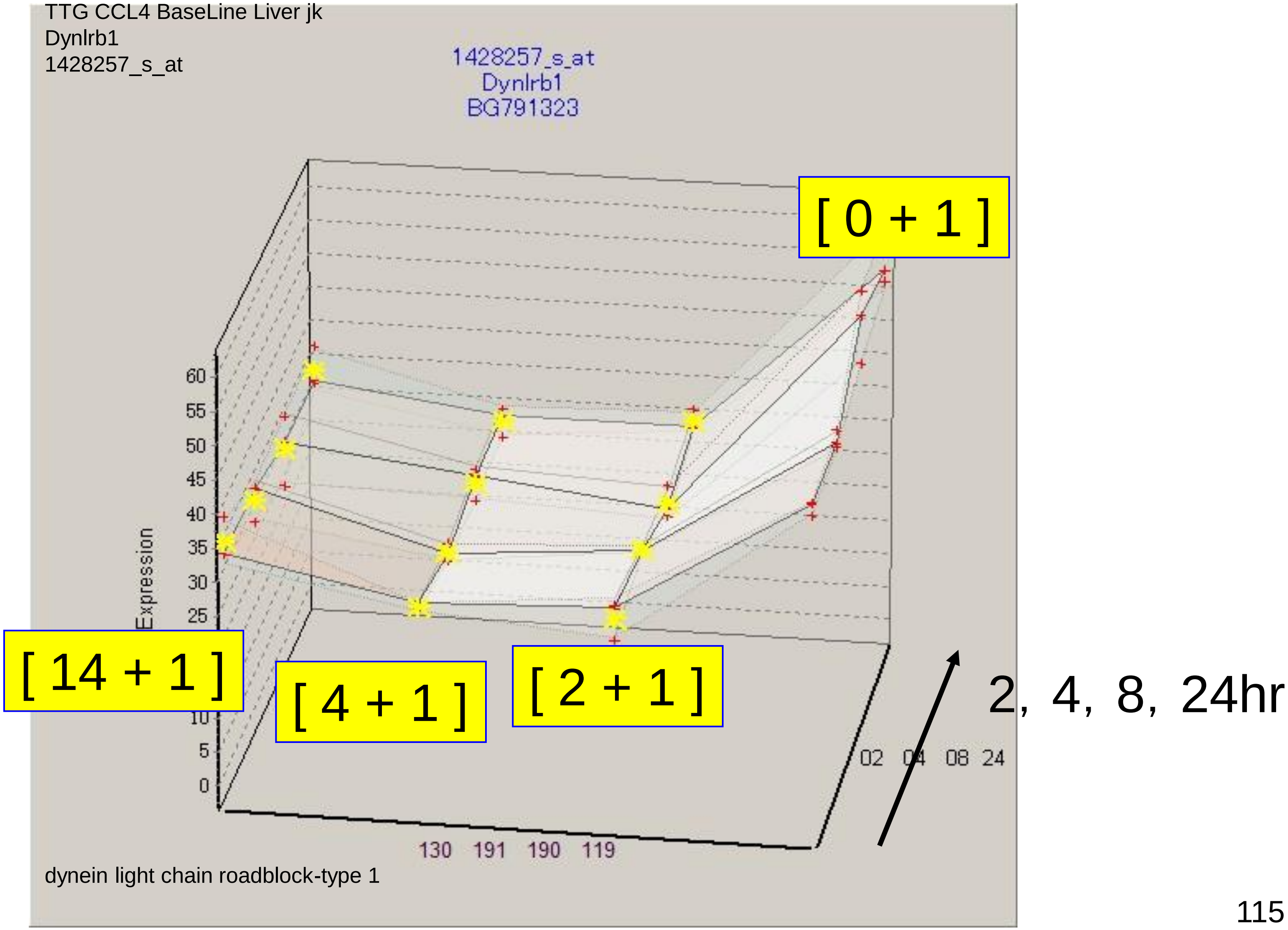
TTG130-L_SpNC_0 1415762_x_at

[14 + 1]



Mrpl52
mitochondrial ribosomal protein L52

A+A’ の Vehicle（溶媒対照群）の比較



115

Unpublished data

A+A’ protocol revealed,,,,

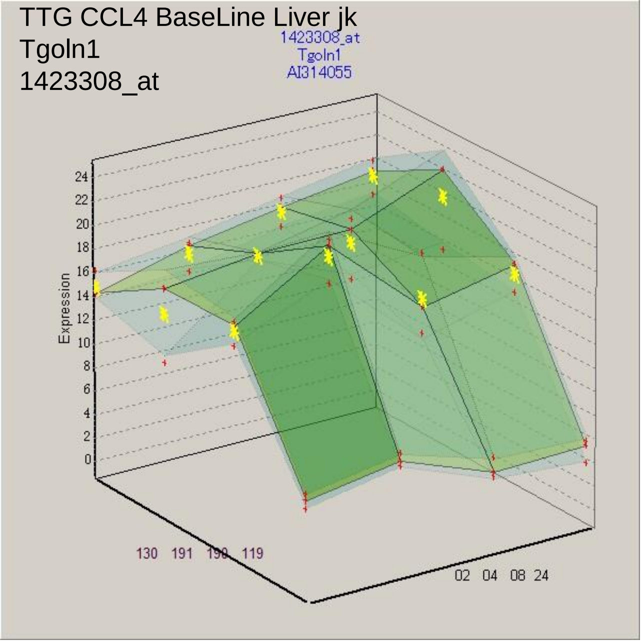
■【B-Res-dominant chemical】

Baseline goes down and T-Res gets suppressed
Baseline goes up and T-Res gets enhanced

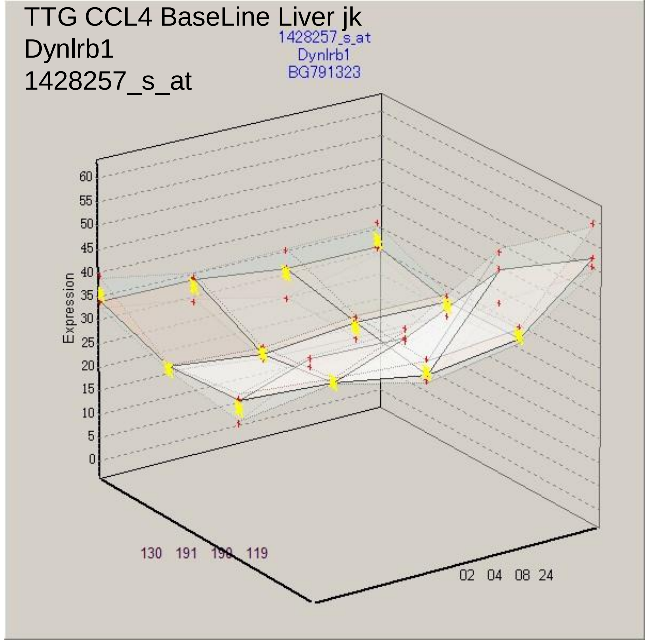
★ How Baseline changes by Repeated dose
・Time-lapse data

★★ What is upstream of B-Res changes; up/ down (unchanged)

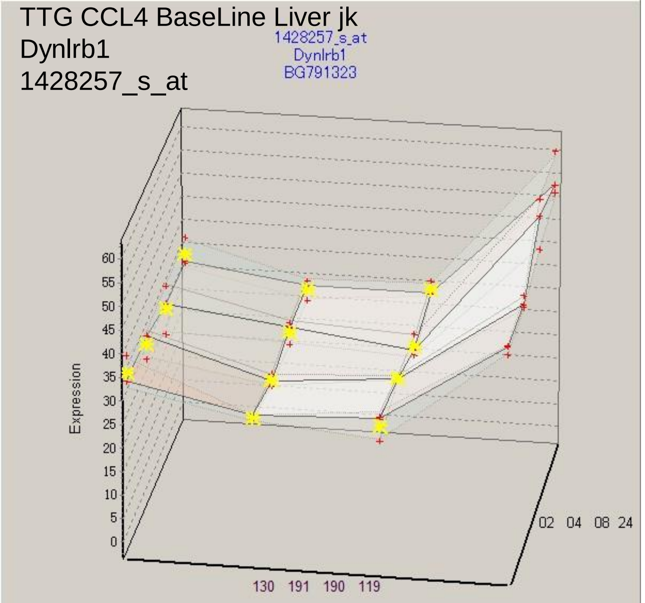
★★★ Does B-Res and T-Res apply for B of the A+B Protocol



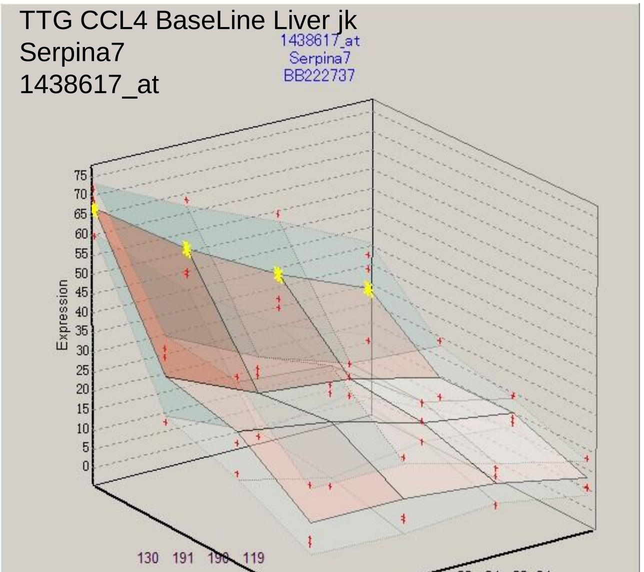
trans-golgi network protein



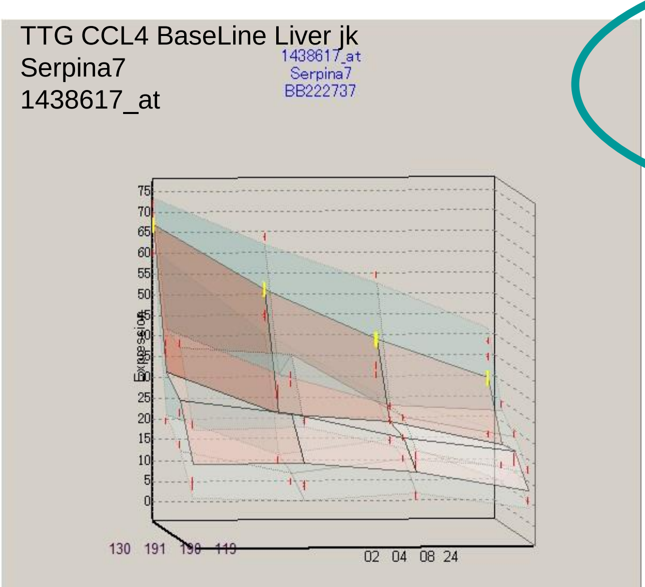
dynein light chain roadblock-type 1



dynein light chain roadblock-type 1



serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7

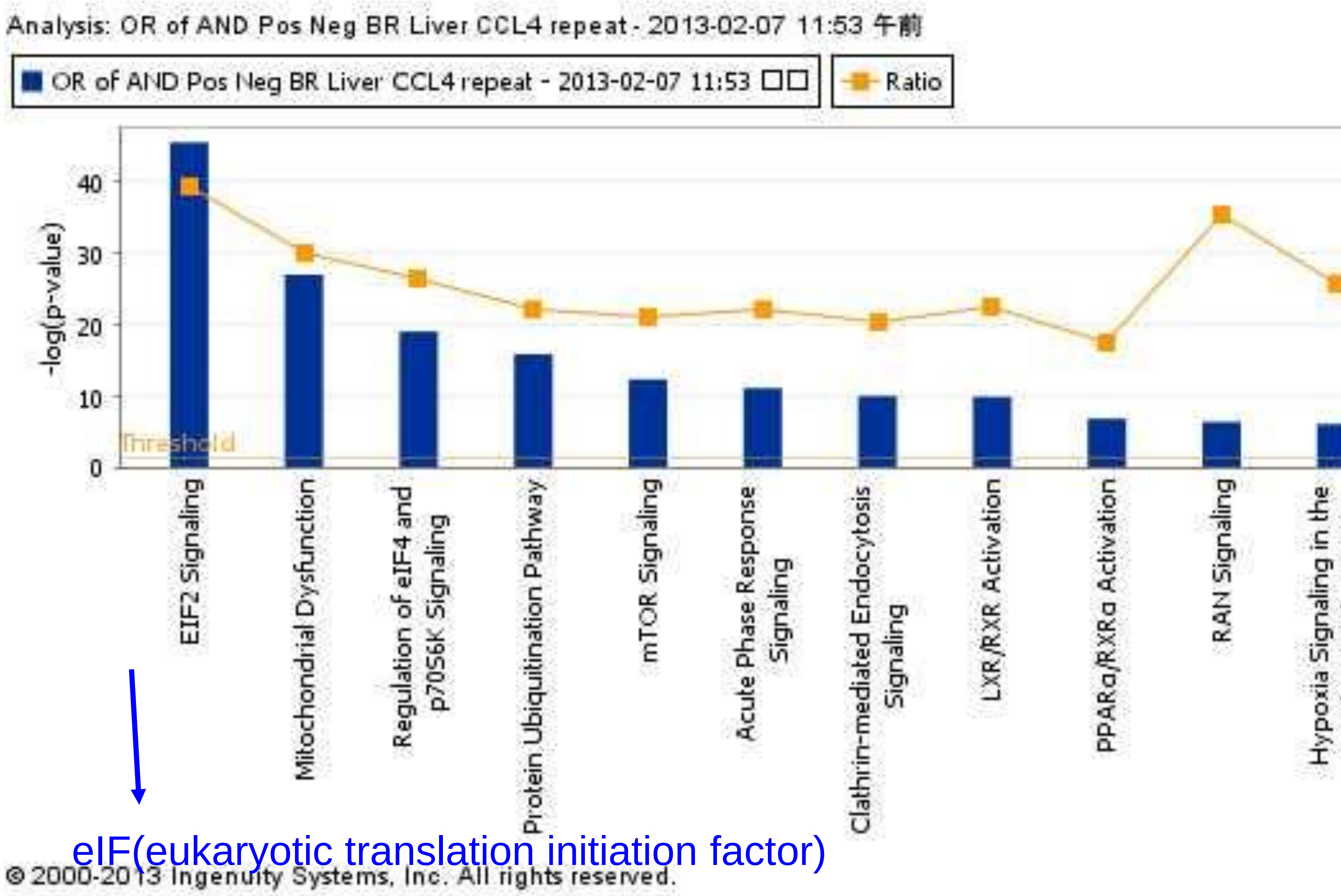


serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7

B-Res Up :ca. 1000 ps
B-Res Dn :ca. 1300 ps

∨

↑ : 219 ps
↓ : 20 ps
→ : 8 ps



eIF(eukaryotic translation initiation factor)

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What is “repeated dose”

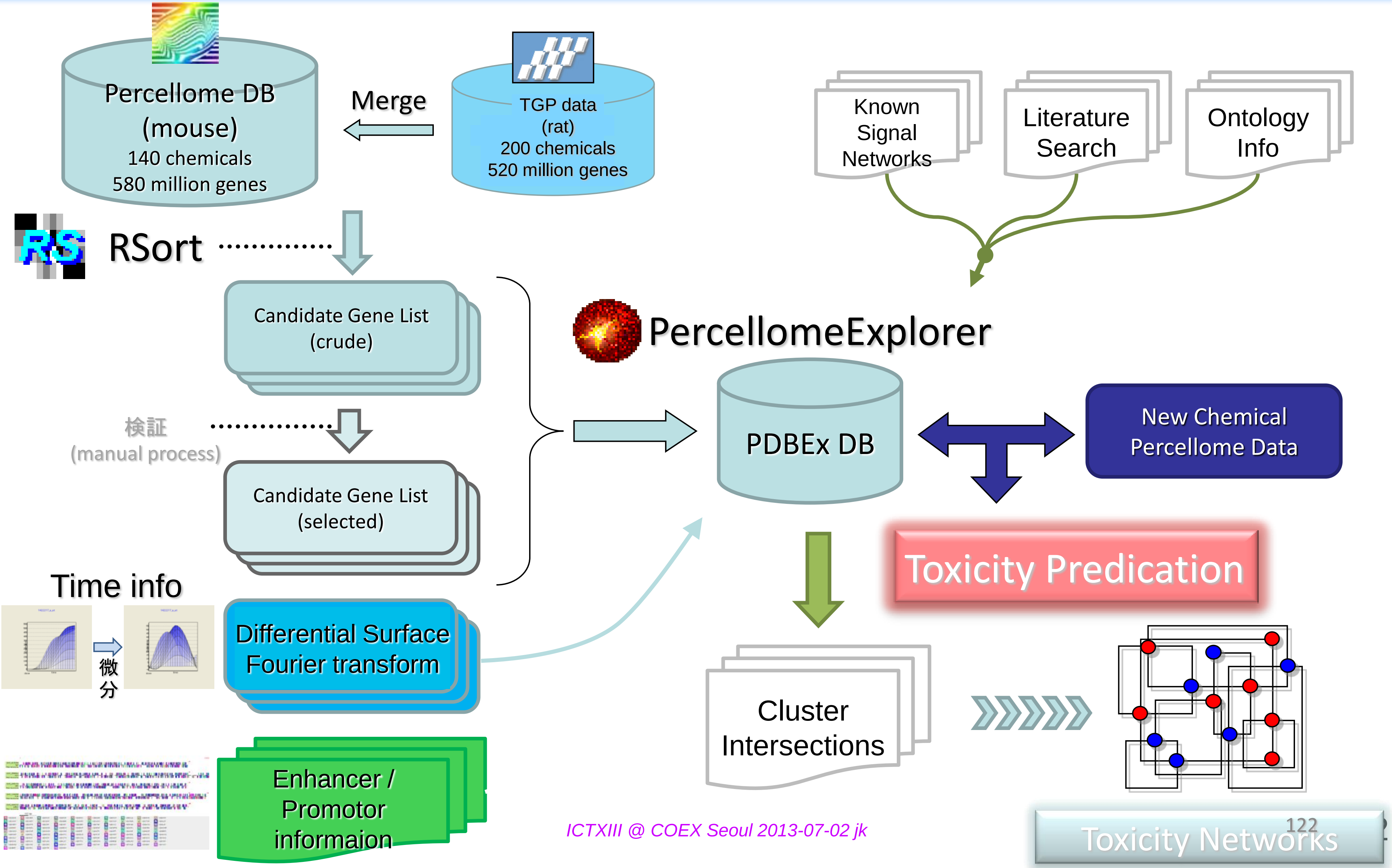
- Epigenetic control of Baseline/Transient Response
  - DNA methylation?
  - Histone modification?

★Upstream of epigenetic control

- EPIGENETIC TOXICITY (named by Dr. Kitano)

Publication of Data

Percellome data flow towards Toxicity Prediction



Percellome DB (mouse)
140 chemicals
580 million genes

Merge

TGP data (rat)
200 chemicals
520 million genes

RSort

Candidate Gene List (crude)

検証
(manual process)

Candidate Gene List (selected)

Time info

微分

Differential Surface Fourier transform

Enhancer / Promotor informaion

Known Signal Networks

Literature Search

Ontology Info

PercellomeExplorer

PDBEx DB

New Chemical Percellome Data

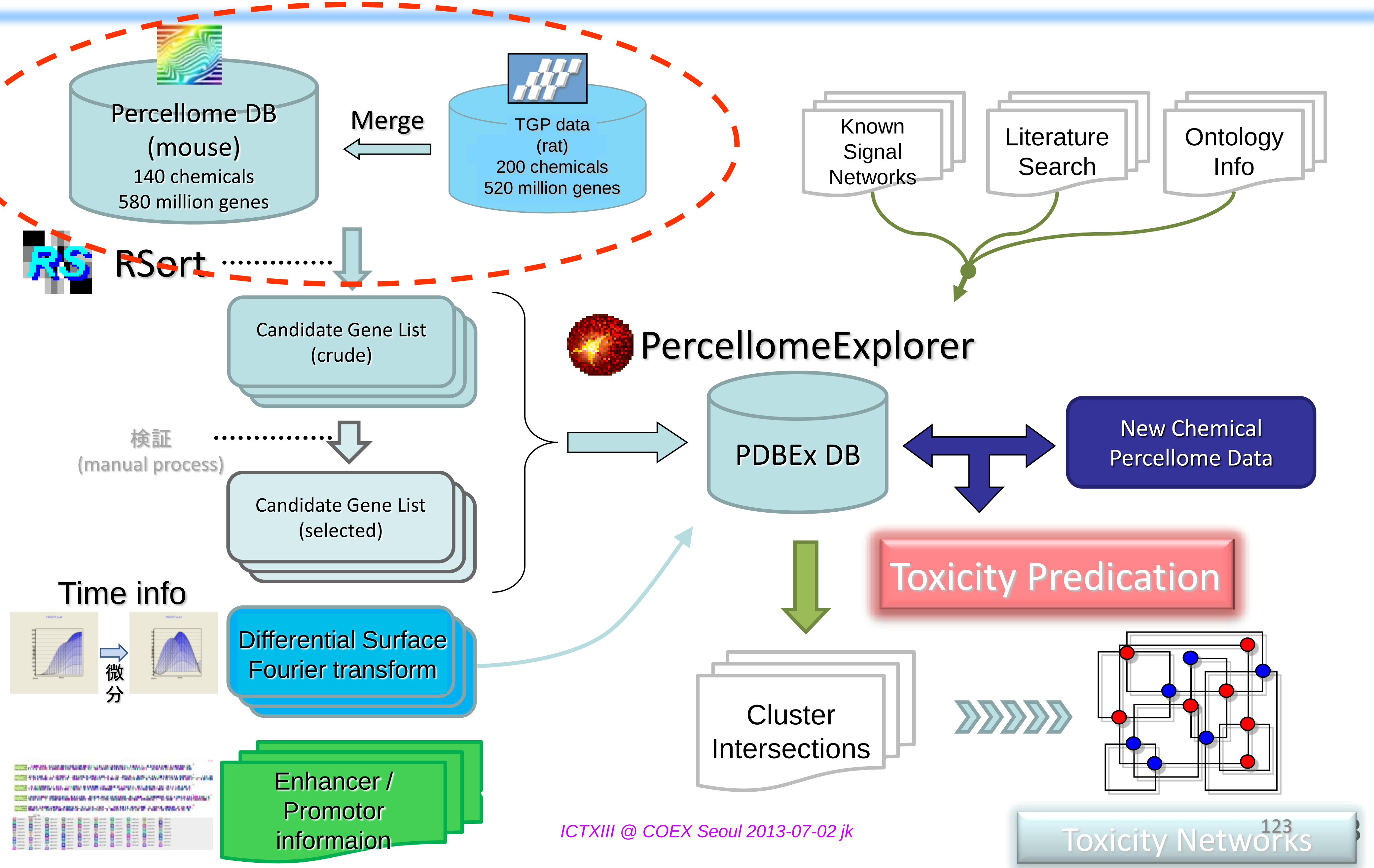
Toxicity Predication

Cluster Intersections

Toxicity Networks

ICTXIII @ COEX Seoul 2013-07-02 jk

Percellome data flow towards Toxicity Prediction



Percellome DB
(mouse)
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Candidate Gene List
(crude)

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(manual process)

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(selected)

PercellomeExplorer

PDBEx DB

New Chemical Percellome Data

Toxicity Predication

Time info

微分

Differential Surface Fourier transform

Enhancer / Promotor informaion

Cluster Intersections

Toxicity Networks

ICTXIII @ COEX Seoul 2013-07-02 jk

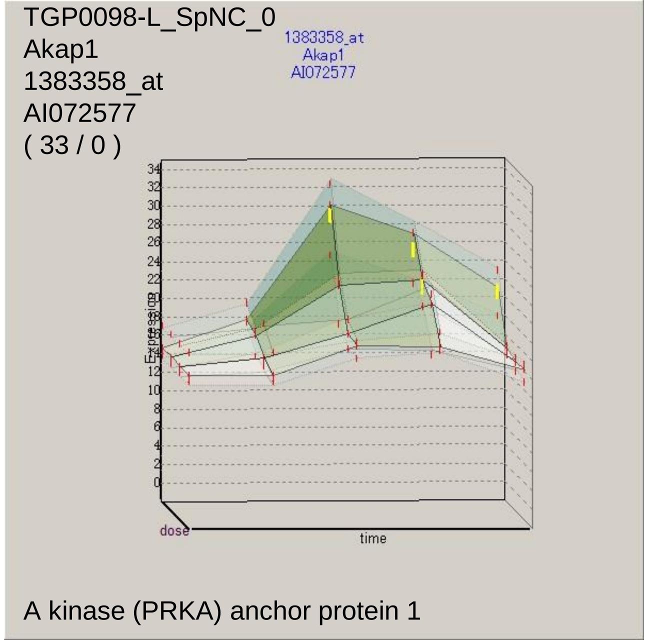
TGP rat data union

- TG-Gates data are Designed as Percellome data
- 3-D data expression, RSort, PercellomeExplorer can be applied
- Rat Gene ID <> Mouse Gene ID connection by Gene common name (many-to-many correspondence)
- R-M converter, M-R converter

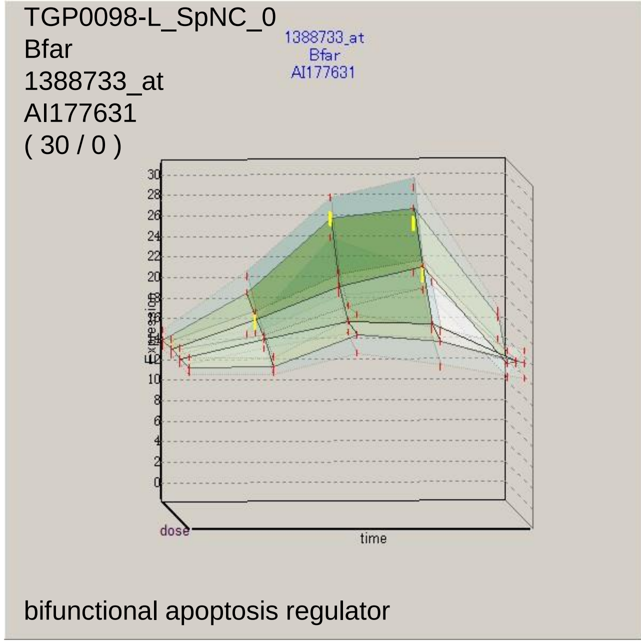
example

Clofibrate

Single oral
to
RAT
(3, 6, 9, 24hr)


TGP0098-L_SpNC_0
Akap1
1383358_at
AI072577
(33 / 0)

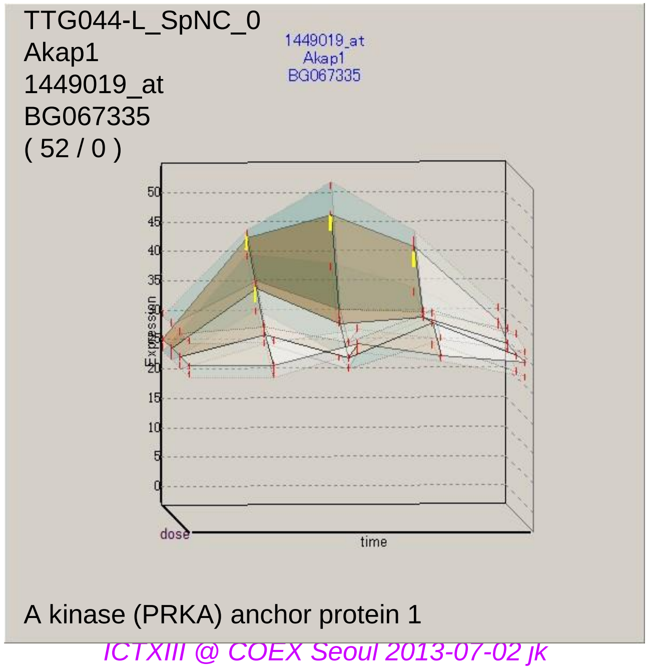
A kinase (PRKA) anchor protein 1


TGP0098-L_SpNC_0
Bfar
1388733_at
AI177631
(30 / 0)

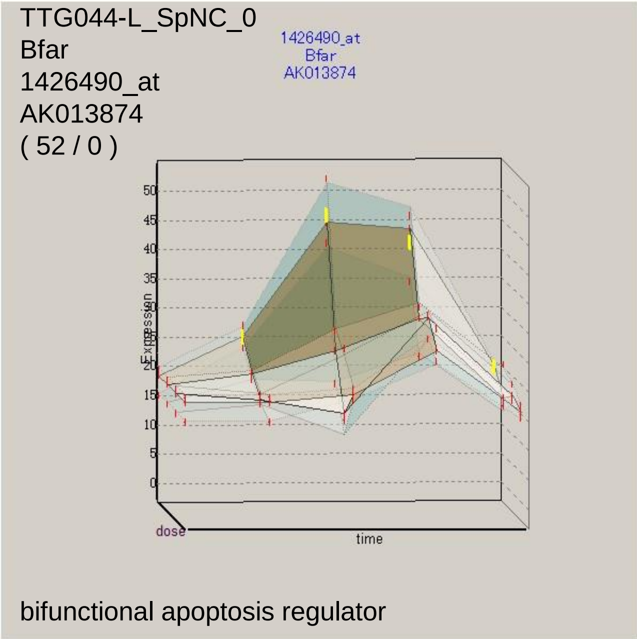
bifunctional apoptosis regulator

Clofibrate

Single oral
to
MOUSE
(2, 4, 8, 24hr)


TTG044-L_SpNC_0
Akap1
1449019_at
BG067335
(52 / 0)

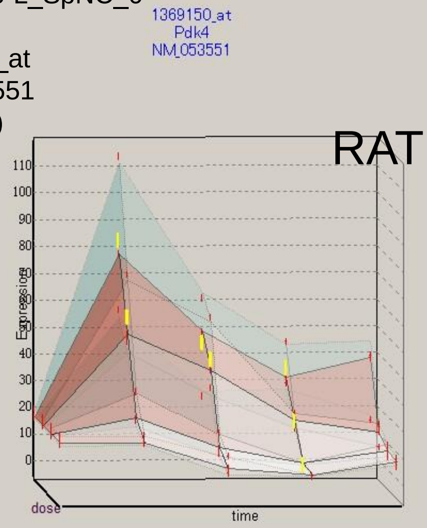
A kinase (PRKA) anchor protein 1


TTG044-L_SpNC_0
Bfar
1426490_at
AK013874
(52 / 0)

bifunctional apoptosis regulator

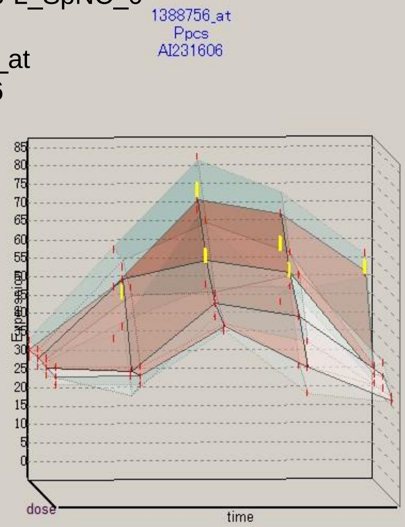
TGP0098-L_SpNC_0
Pdk4
1369150_at
NM_053551
(114 / 0)

RAT



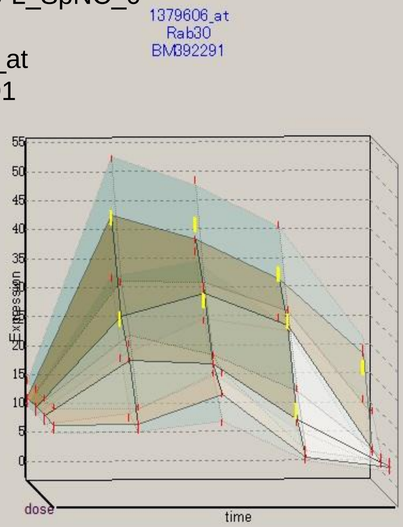
pyruvate dehydrogenase kinase, isozyme 4

TGP0098-L_SpNC_0
Ppcs
1388756_at
AI231606
(82 / 0)



phosphopantothenoylcysteine synthetase

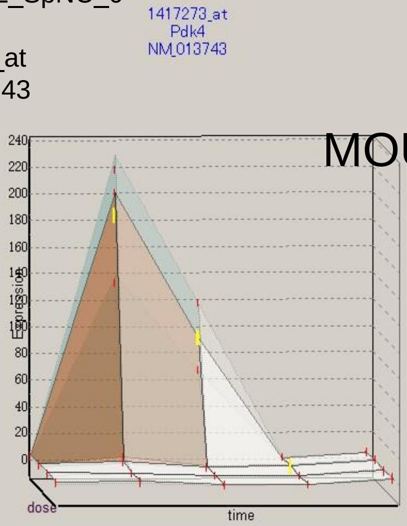
TGP0098-L_SpNC_0
Rab30
1379606_at
BM392291
(53 / 0)



RAB30, member RAS oncogene family

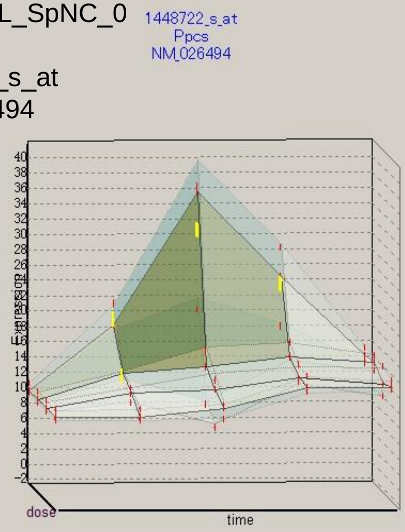
TTG044-L_SpNC_0
Pdk4
1417273_at
NM_013743
(229 / 0)

MOUSE



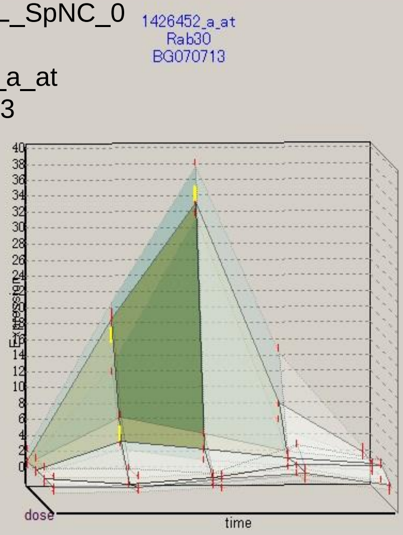
pyruvate dehydrogenase kinase, isoenzyme 4

TTG044-L_SpNC_0
Ppcs
1448722_s_at
NM_026494
(40 / 0)



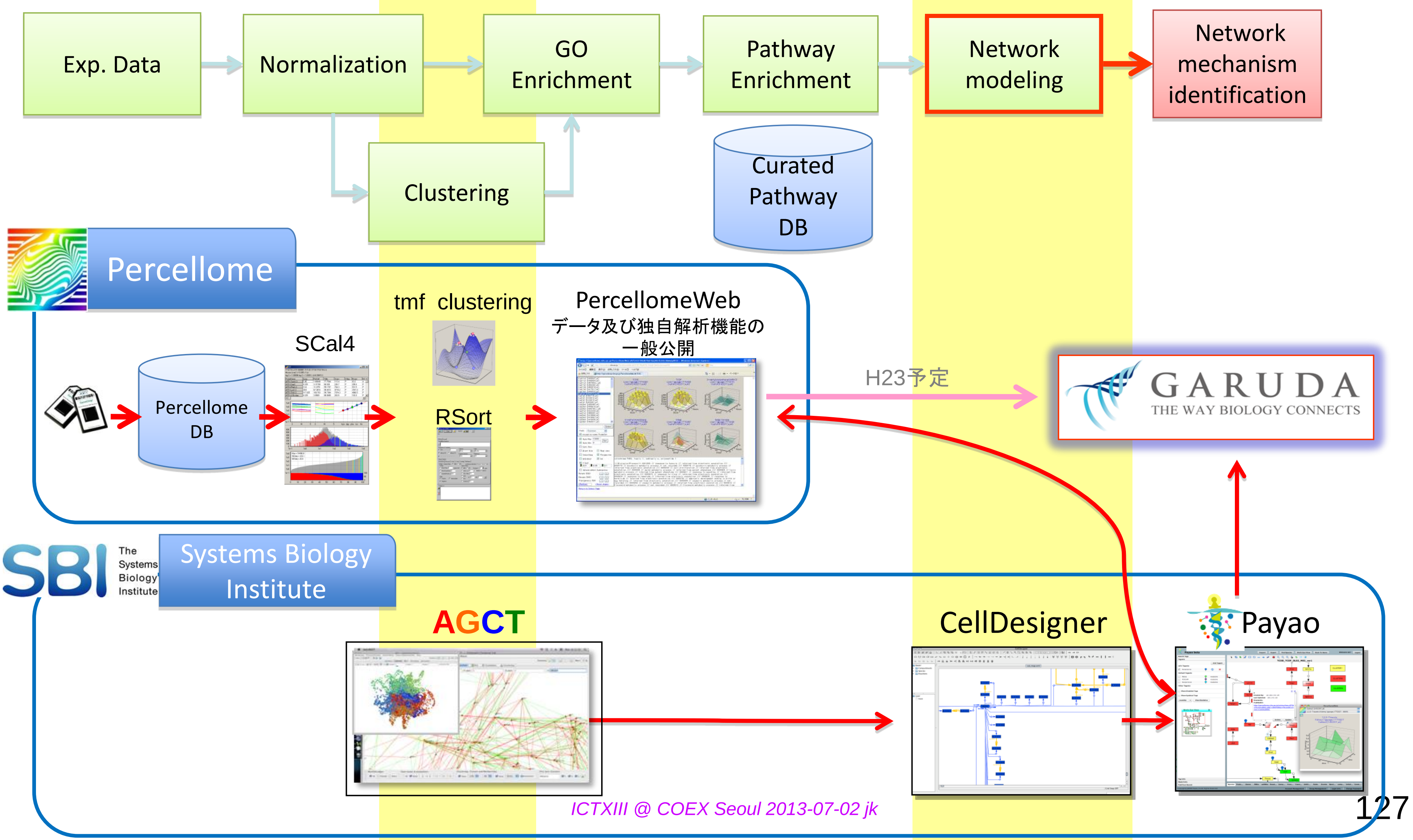
phosphopantothenoylcysteine synthetase

TTG044-L_SpNC_0
Rab30
1426452_a_at
BG070713
(38 / 0)



RAB30, member RAS oncogene family

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



Exp. Data → Normalization → GO Enrichment → Pathway Enrichment → Network modeling → Network mechanism identification

Normalization → Clustering → GO Enrichment

Curated Pathway DB

Percellome

Percellome DB → SCal4 → tmf clustering / RSort → PercellomeWeb データ及び独自解析機能の一般公開

H23予定

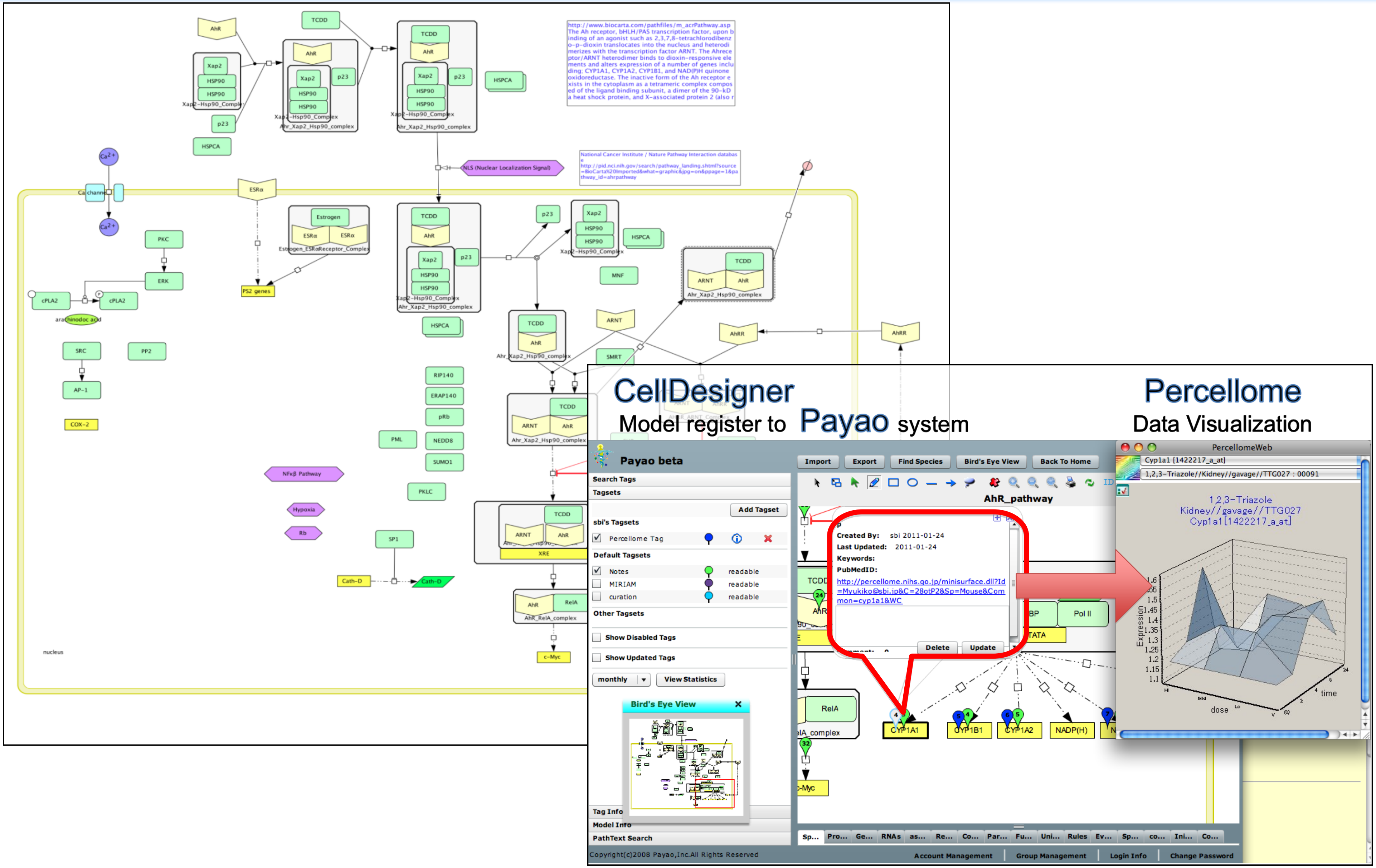
GARUDA
THE WAY BIOLOGY CONNECTS

The Systems Biology Institute

Systems Biology Institute

AGCT → CellDesigner → Payao

AhR pathway built by CellDesigner and Payao


AhR
TCDD
Xap2
HSP90
p23
HSPCA
Xap2-Hsp90_Complex
Ahr_Xap2_Hsp90_complex
NLS (Nuclear Localization Signal)
Ca2+
Ca channel
ESRα
Estrogen
Estrogen_ESRαReceptor_Complex
PS2 genes
PKC
ERK
cPLA2
arachinodoc acid
SRC
PP2
AP-1
COX-2
MNF
ARNT
AhRR
SMRT
RIP140
ERAP140
pRb
PML
NEDD8
SUMO1
PKLC
NFκB Pathway
Hypoxia
Rb
SP1
Cath-D
XRE
RelA
AhR_RelA_complex
c-Myc
nucleus
Payao beta
Search Tags
Tagsets
Add Tagset
sbi's Tagsets
☑ Percellome Tag
Default Tagsets
☑ Notes readable
☐ MIRIAM readable
☐ curation readable
Other Tagsets
☐ Show Disabled Tags
☐ Show Updated Tags
monthly
View Statistics
Bird's Eye View
Tag Info
Model Info
PathText Search
Import
Export
Find Species
Bird's Eye View
Back To Home
AhR_pathway
Delete
Update
TATA
Pol II
CYP1A1
CYP1B1
CYP1A2
NADP(H)
Sp... Pro... Ge... RNAs as... Re... Co... Par... Fu... Uni... Rules Ev... Sp... co... Ini... Co...
Copyright(c)2008 Payao,Inc.All Rights Reserved
Account Management
Group Management
Login Info
Change Password
PercellomeWeb
Cyp1a1 [1422217_a_at]
1,2,3-Triazole//Kidney//gavage//TTG027 : 00091
1,2,3-Triazole
Kidney//gavage//TTG027
Cyp1a1[1422217_a_at]
Expression
dose
time

http://www.biocarta.com/pathfiles/m_acrPathway.asp
The Ah receptor, bHLH/PAS transcription factor, upon binding of an agonist such as 2,3,7,8-tetrachlorodibenzo-p-dioxin translocates into the nucleus and heterodimerizes with the transcription factor ARNT. The Ahreceptor/ARNT heterodimer binds to dioxin-responsive elements and alters expression of a number of genes including; CYP1A1, CYP1A2, CYP1B1, and NAD(P)H quinone oxidoreductase. The inactive form of the Ah receptor exists in the cytoplasm as a tetrameric complex composed of the ligand binding subunit, a dimer of the 90-kD a heat shock protein, and X-associated protein 2 (also r

National Cancer Institute / Nature Pathway Interaction database
http://pid.nci.nih.gov/search/pathway_landing.shtml?source=BioCarta%20Imported&what=graphic&jpg=on&ppage=1&pathway_id=ahrpathway

Created By: sbi 2011-01-24
Last Updated: 2011-01-24
Keywords:
PubMedID:
http://percellome.nihs.go.jp/minisurface.dll?Id=Myukiko@sbi.jp&C=28otP2&Sp=Mouse&Common=cyp1a1&WC

CellDesigner
Model register to **Payao** system

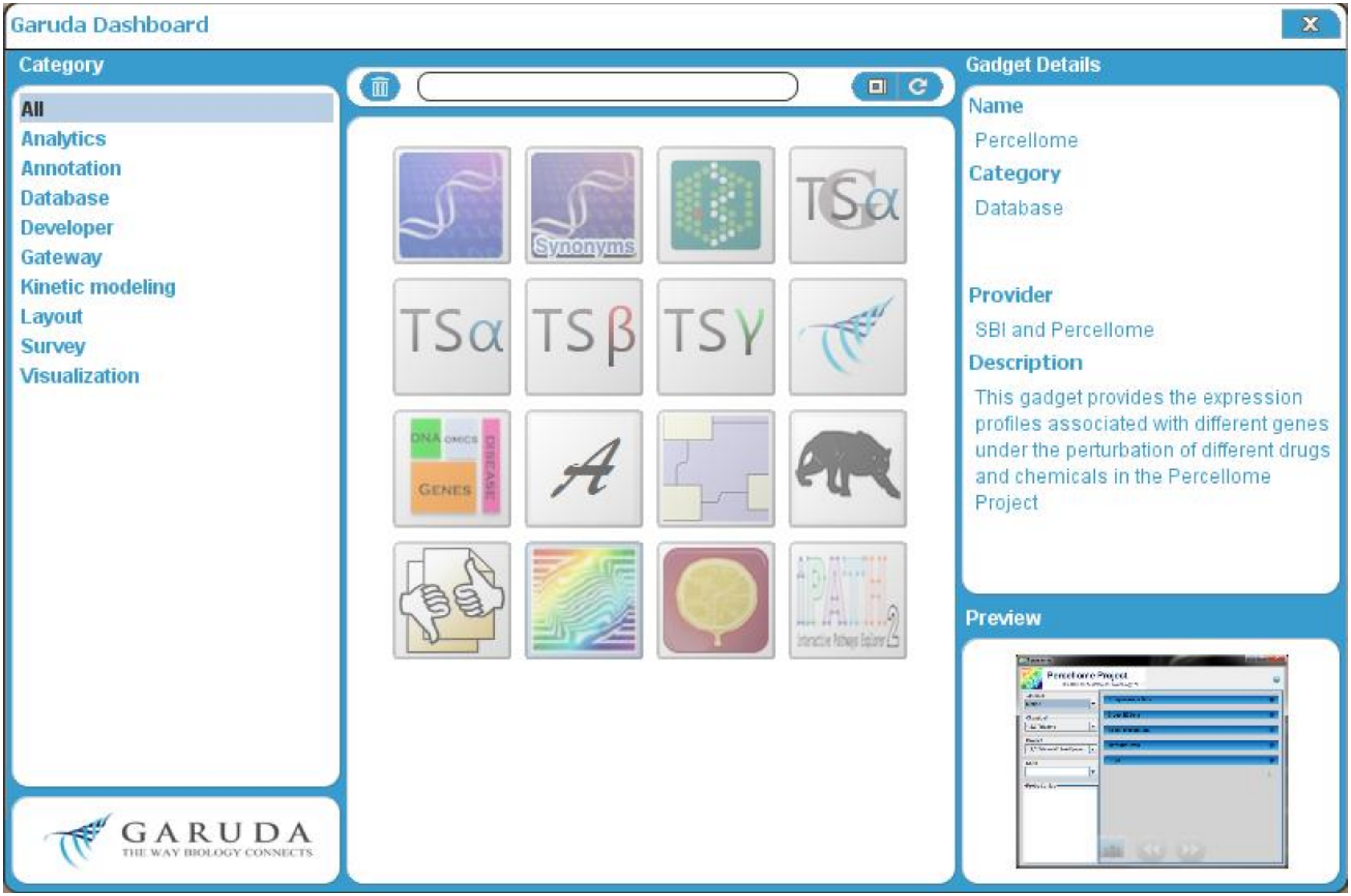
Percellome
Data Visualization

Garuda: Aug 2013 open

(a part of OPEN BIOLOGY)

Pre-alpha Alliance Demo

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


Garuda Dashboard
Category
All
Analytics
Annotation
Database
Developer
Gateway
Kinetic modeling
Layout
Survey
Visualization
Synonyms
TSα
TSβ
TSγ
DNA
OMICS
GENES
DISEASE
iPATH2
GARUDA
THE WAY BIOLOGY CONNECTS
Gadget Details
Preview

Name

Percellome

Category

Database

Provider

SBI and Percellome

Description

This gadget provides the expression profiles associated with different genes under the perturbation of different drugs and chemicals in the Percellome Project

Summary (Percellome)

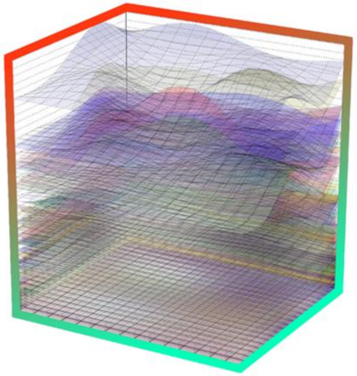
- Ready to Draw dynamic networks with time course
  - Combination study, etc.
- Merging rat TGP data to mouse Percellome data
  - In PercellomeExplorer
- Publish all data to public domain along with all analysis tools via Garuda
  - Accelerate/ Synergize analysis speed by world wide users

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



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

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,
Bun-ichi Tajima)

Neon Swan

Back ground

- JSOT organized sessions on

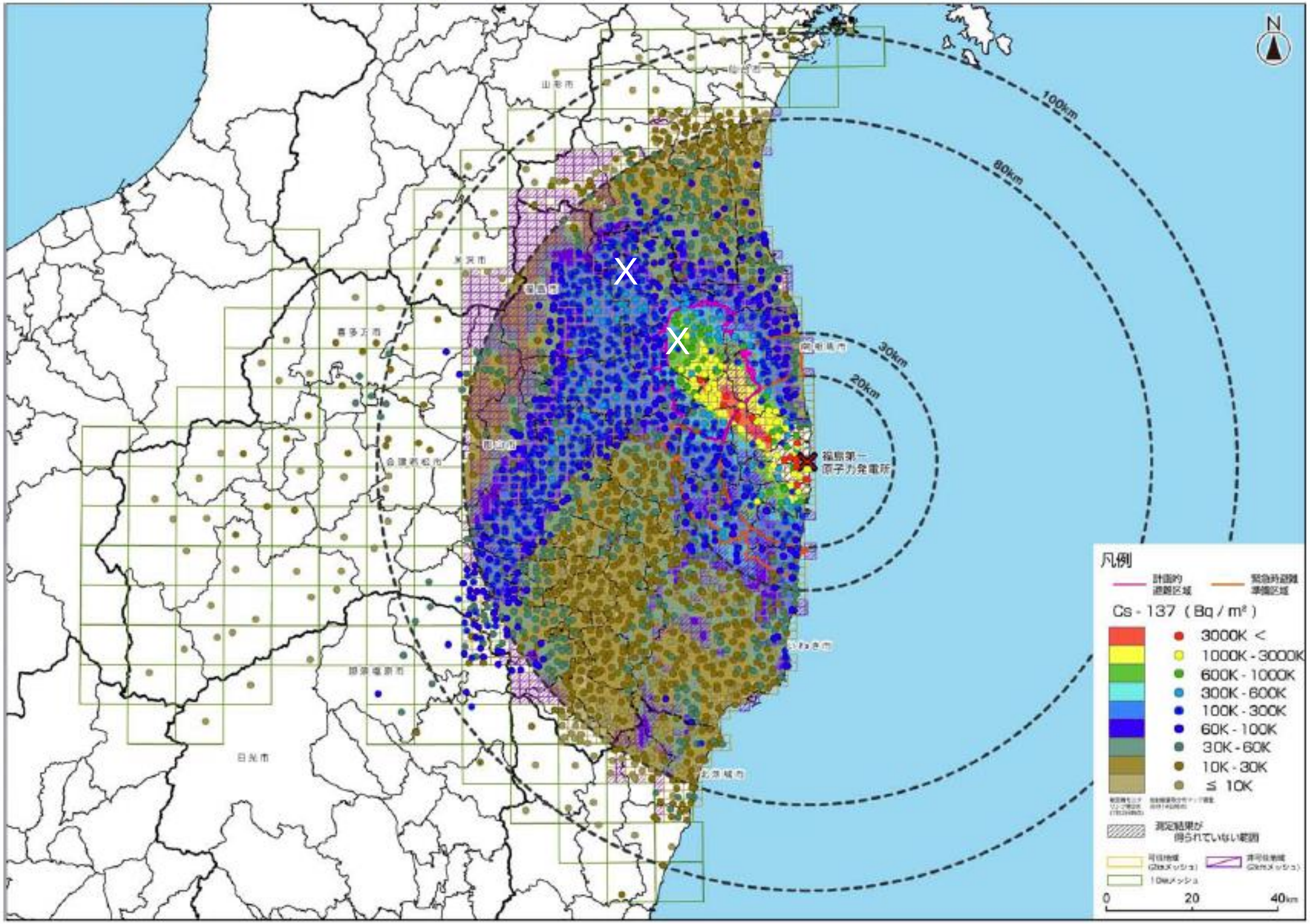
Radiation Toxicology

after the incidence.

Because,,,,

第3次航空機モニタリング結果とセシウム137の土壌濃度マップの比較について

別紙6


N
100km
80km
30km
20km
X
X
山形市
仙台市
米沢市
福島市
喜多方市
郡山市
会津若松市
南相馬市
福島第一原子力発電所
いわき市
那須塩原市
日光市
北茨城市
凡例
計画的避難区域
緊急時避難準備区域
Cs - 137 （Bq / m²）
3000K <
1000K - 3000K
600K - 1000K
300K - 600K
100K - 300K
60K - 100K
30K - 60K
10K - 30K
≦ 10K
測定結果が得られていない範囲
可住地域（2kmメッシュ）
非可住地域（2kmメッシュ）
10kmメッシュ
0
20
40km

VSD (virtually safe dose)

Chemicals

- 10^{-6}
- 10^{-5}

Aflatoxins

- 10^{-4}～ 10^{-3} Asbestos (occupational)
  - In Japan, mesothelioma patient is 1,000 per year, with unknown numbers of lung cancer.

Radiation

- 10^{-2} 100mSv (threshold anounced by Japanese FSC*)

if dose rate effect is considered,

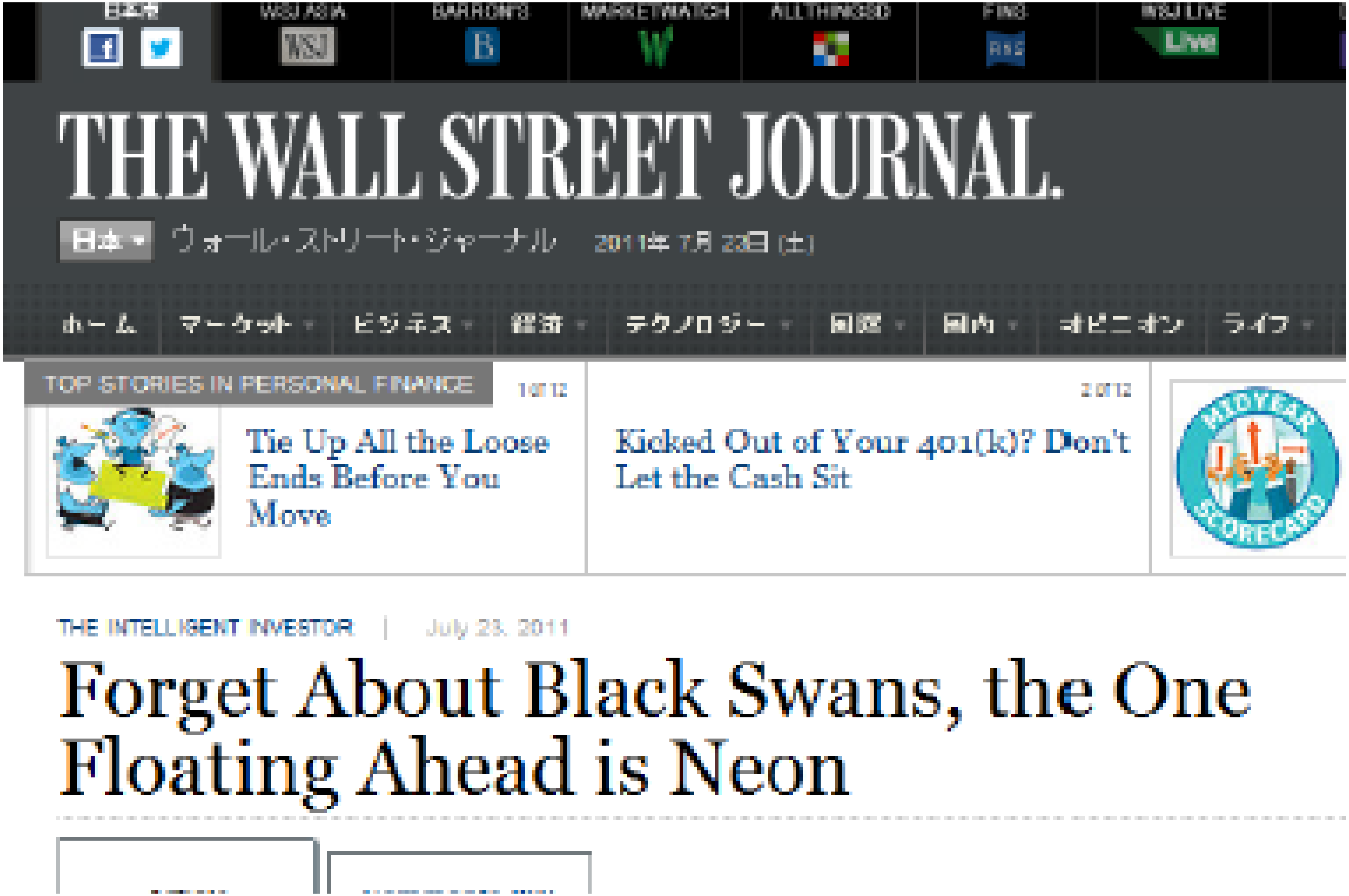
DDREF 100mSv = 10^{-4} ?

FSC: Food Safety Commission of Japan

- A talk was made by our collaborator, Dr. Hiroaki Kitano, Systems Biology Institute, Tokyo, Japan, titled

"Critical View of Op-Eds on Risk of Low-Level Radiation Exposure and Difficulties on Risk Evaluation"

Last topic of his talk was,,,,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MIDYEAR SCORECARD

THE WALL STREET JOURNAL.

ウォール・ストリート・ジャーナル 2011年 7月 23日 (土)

TOP STORIES IN PERSONAL FINANCE

Tie Up All the Loose Ends Before You Move

Kicked Out of Your 401(k)? Don't Let the Cash Sit

THE INTELLIGENT INVESTOR | July 23, 2011

Forget About Black Swans, the One Floating Ahead is Neon

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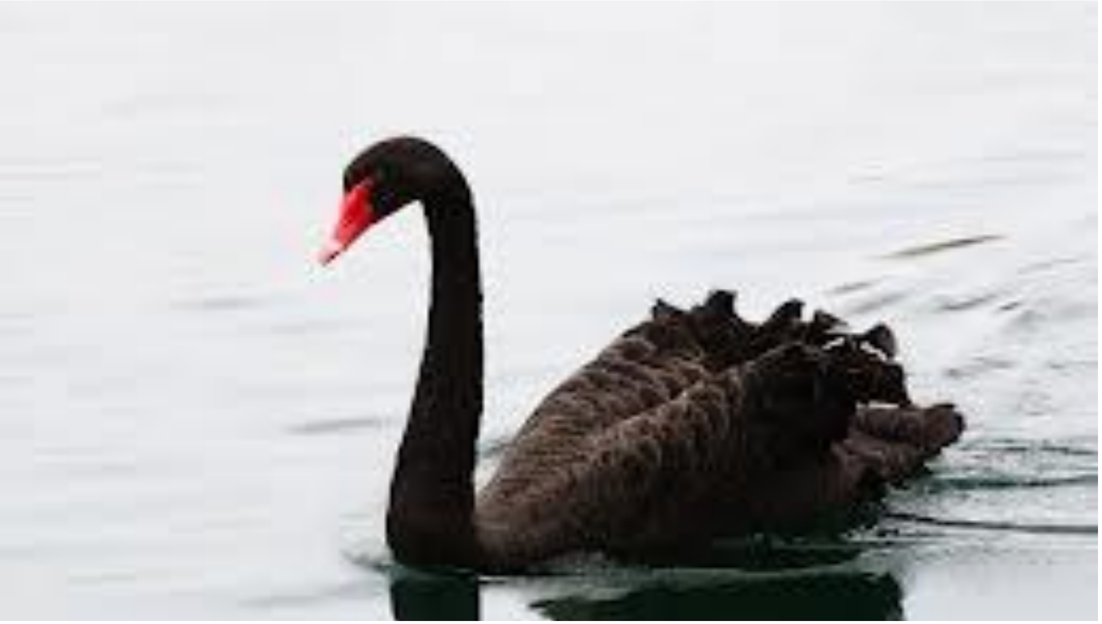
Black Swan

Black Swan



“You've heard of black swans—events that are unthinkably rare, immensely important, and as unpredictable in advance as they are inevitable in hindsight.”

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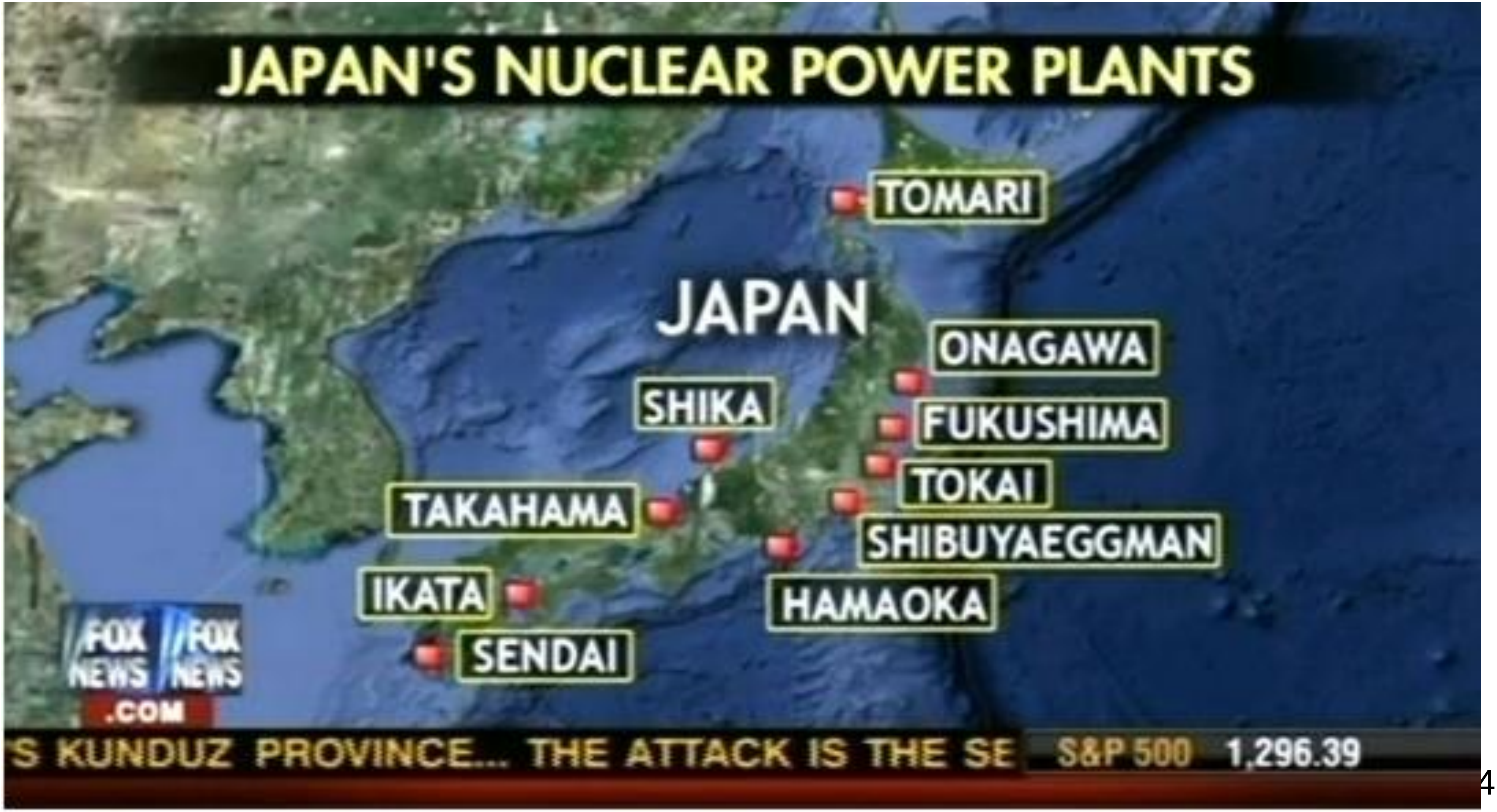
FOX NEWS (March 2011)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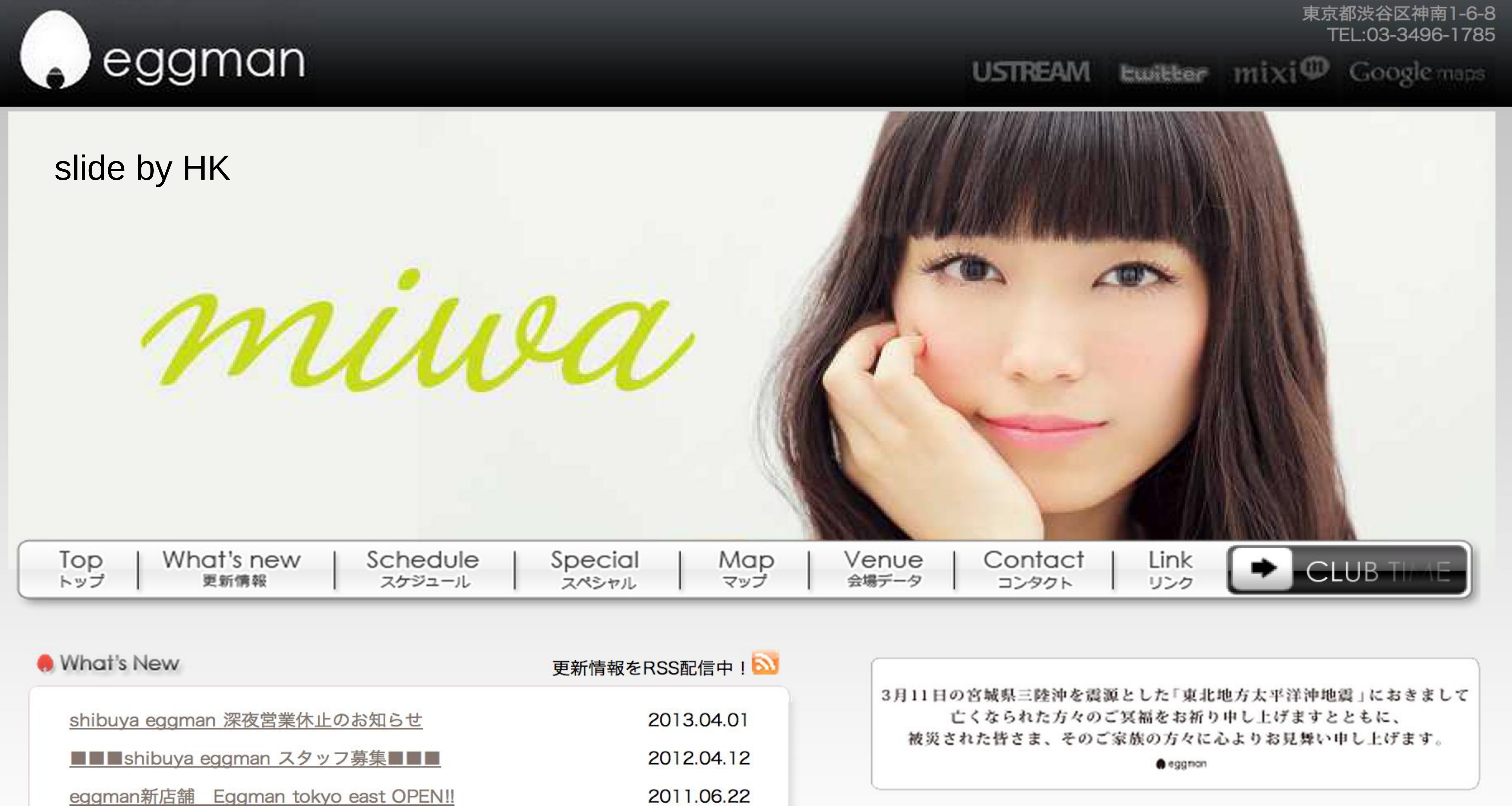
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shibuya eggman 深夜営業休止のお知らせ	2013.04.01
■■■shibuya eggman スタッフ募集■■■	2012.04.12
eggman新店舗　Eggman tokyo east OPEN!!	2011.06.22

3月11日の宮城県三陸沖を震源とした「東北地方太平洋沖地震」におきまして
亡くなられた方々のご冥福をお祈り申し上げますとともに、
被災された皆さま、そのご家族の方々に心よりお見舞い申し上げます。

一部でshibuya eggmanが原子力施設であるという報道がありましたが、全く事実と異なります。
音楽を愛する皆様の心が原動力となっています。
Shibuya Eggman has no nuclear plant. Our electricity's powered only by music.

shibuya eggman　スタッフ一同

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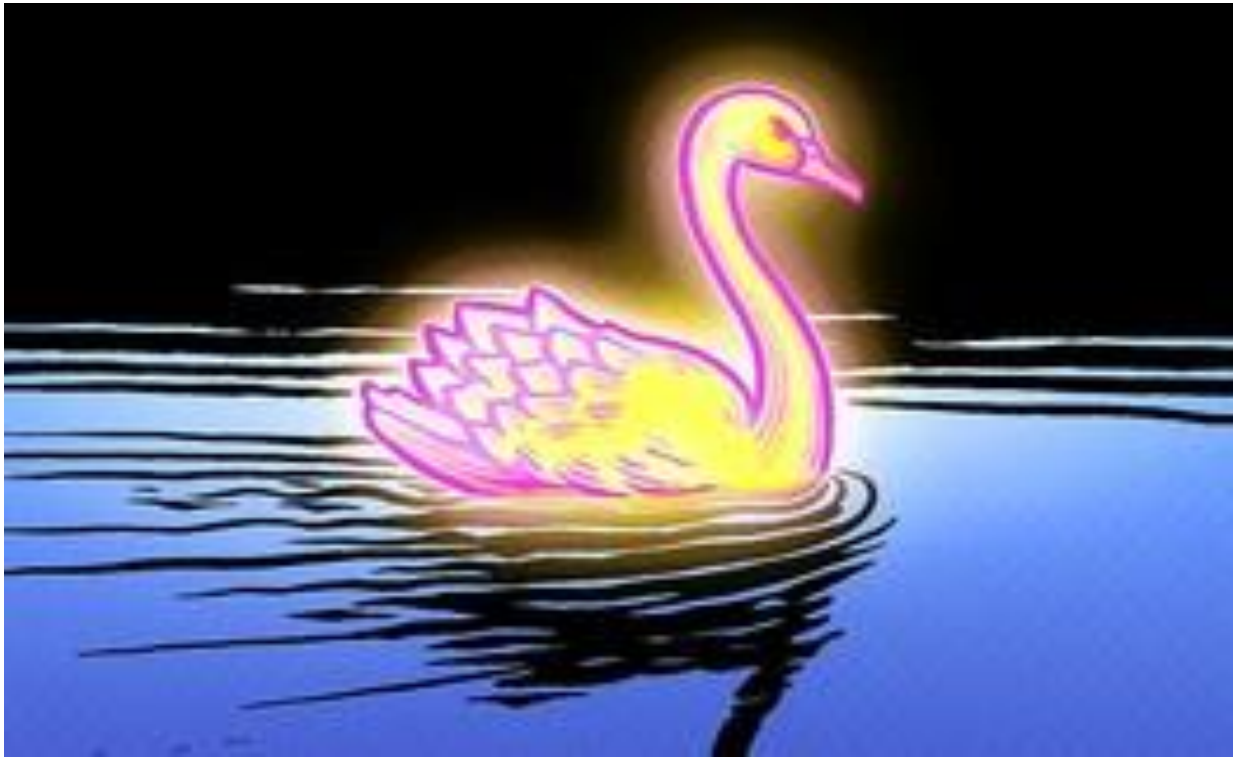
Neon Swan



Neon Swan

“our minds—and our markets—aren't very well equipped to protect us against neon swans, either.

Many investors seem to be coping with what seems like an obvious risk simply by closing their eyes.”

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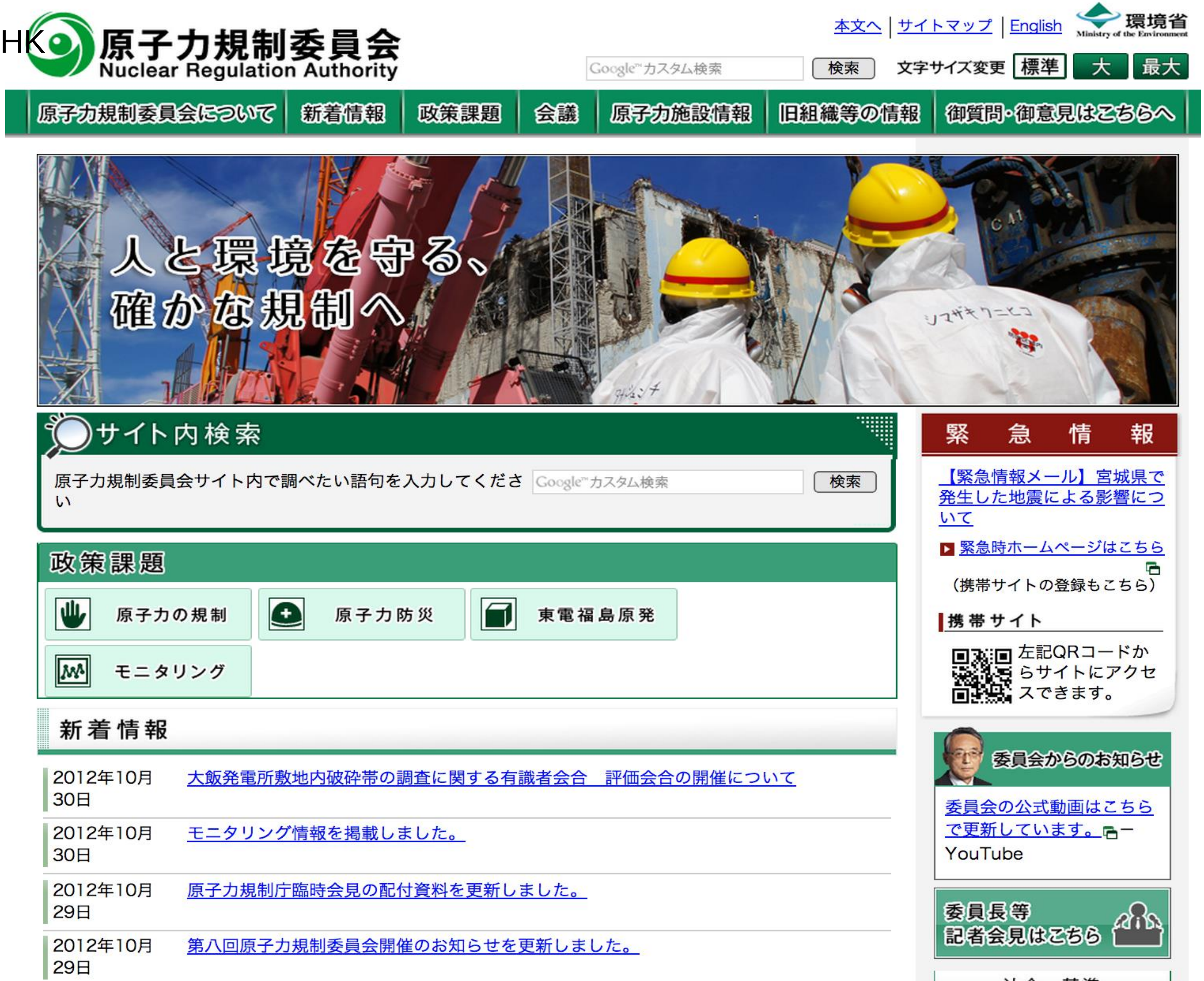


原子力規制委員会
Nuclear Regulation Authority

本文へ | サイトマップ | English

環境省
Ministry of the Environment

Google™カスタム検索 検索

文字サイズ変更 標準 大 最大

原子力規制委員会について | 新着情報 | 政策課題 | 会議 | 原子力施設情報 | 旧組織等の情報 | 御質問・御意見はこちらへ

人と環境を守る、
確かな規制へ

サイト内検索

原子力規制委員会サイト内で調べたい語句を入力してください Google™カスタム検索 検索

政策課題

- 原子力の規制
- 原子力防災
- 東電福島原発
- モニタリング

新着情報

2012年10月30日	大飯発電所敷地内破砕帯の調査に関する有識者会合　評価会合の開催について
2012年10月30日	モニタリング情報を掲載しました。
2012年10月29日	原子力規制庁臨時会見の配付資料を更新しました。
2012年10月29日	第八回原子力規制委員会開催のお知らせを更新しました。

緊急情報

【緊急情報メール】宮城県で発生した地震による影響について

緊急時ホームページはこちら

（携帯サイトの登録もこちら）

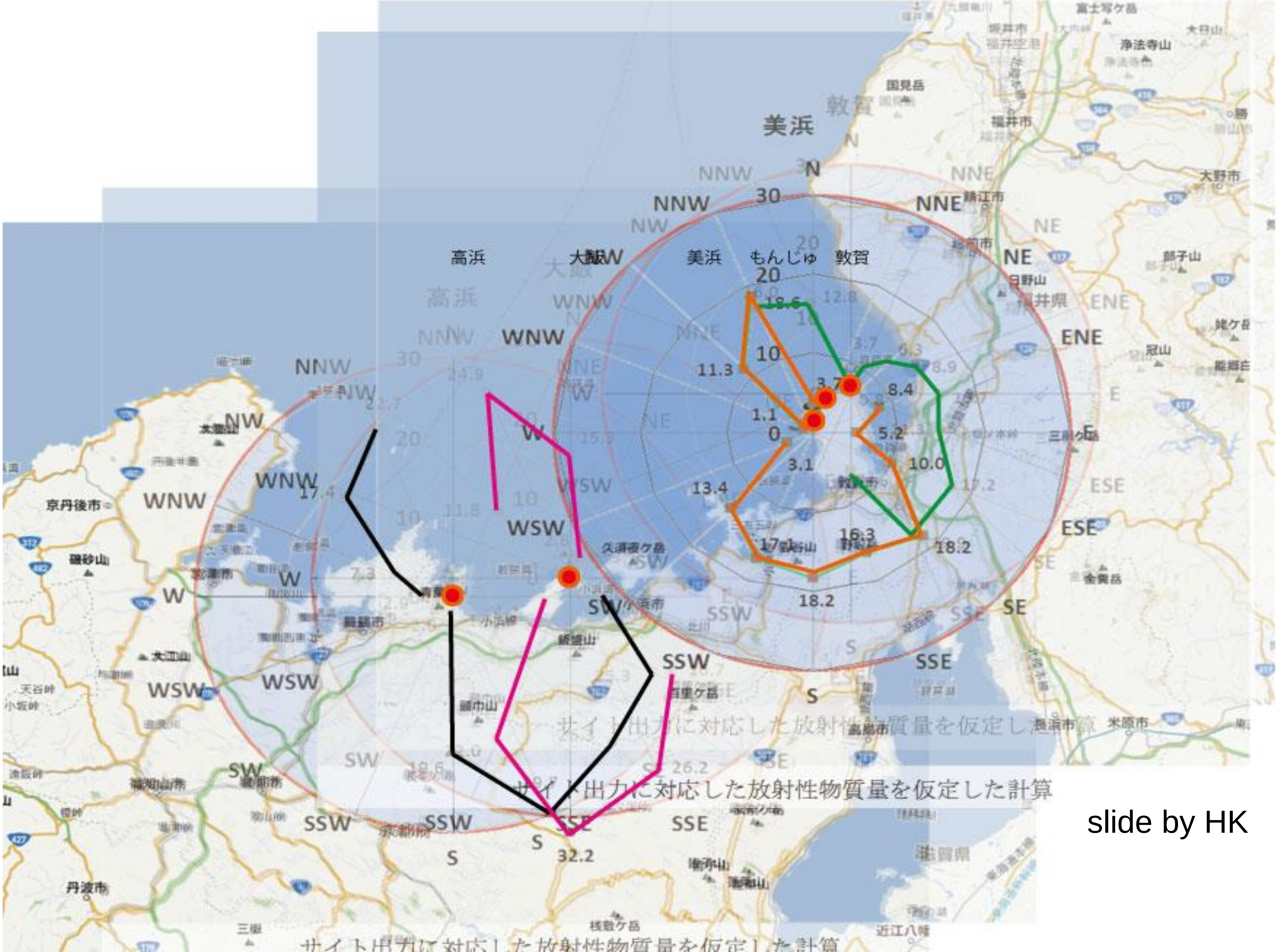
携帯サイト

左記QRコードからサイトにアクセスできます。

委員会からのお知らせ

委員会の公式動画はこちらで更新しています。－YouTube

委員長等記者会見はこちら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32.2
サイト出力に対応した放射性物質量を仮定した計算

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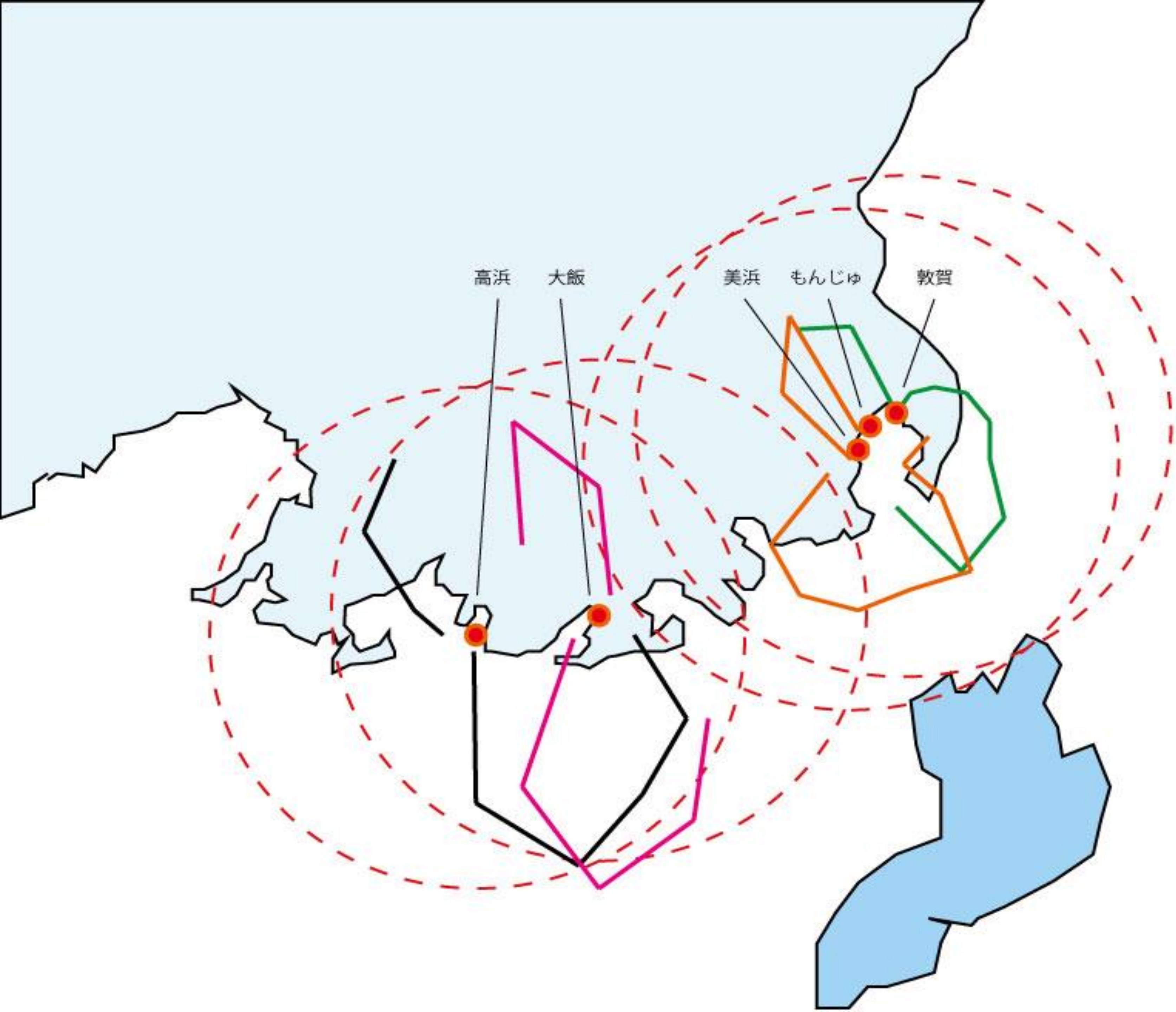
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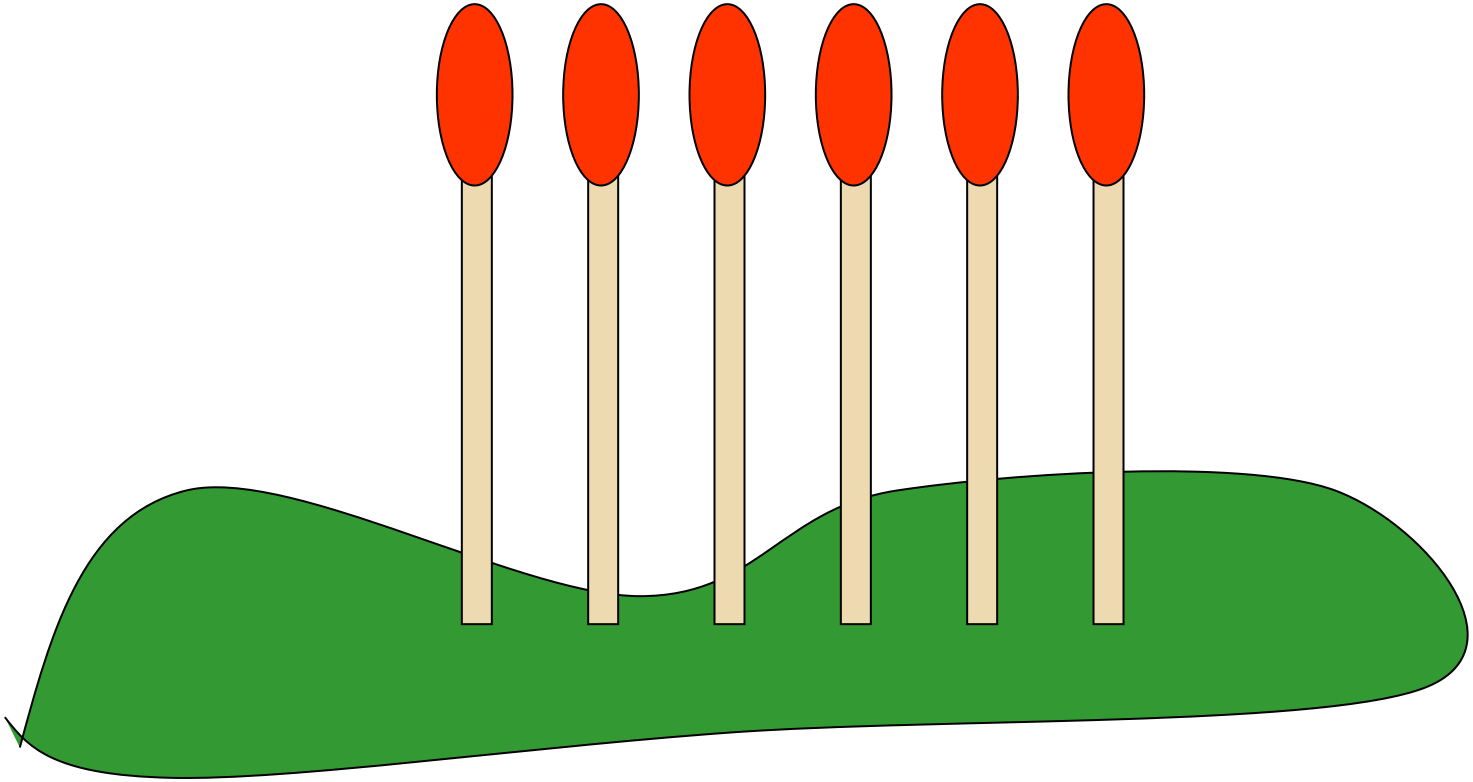

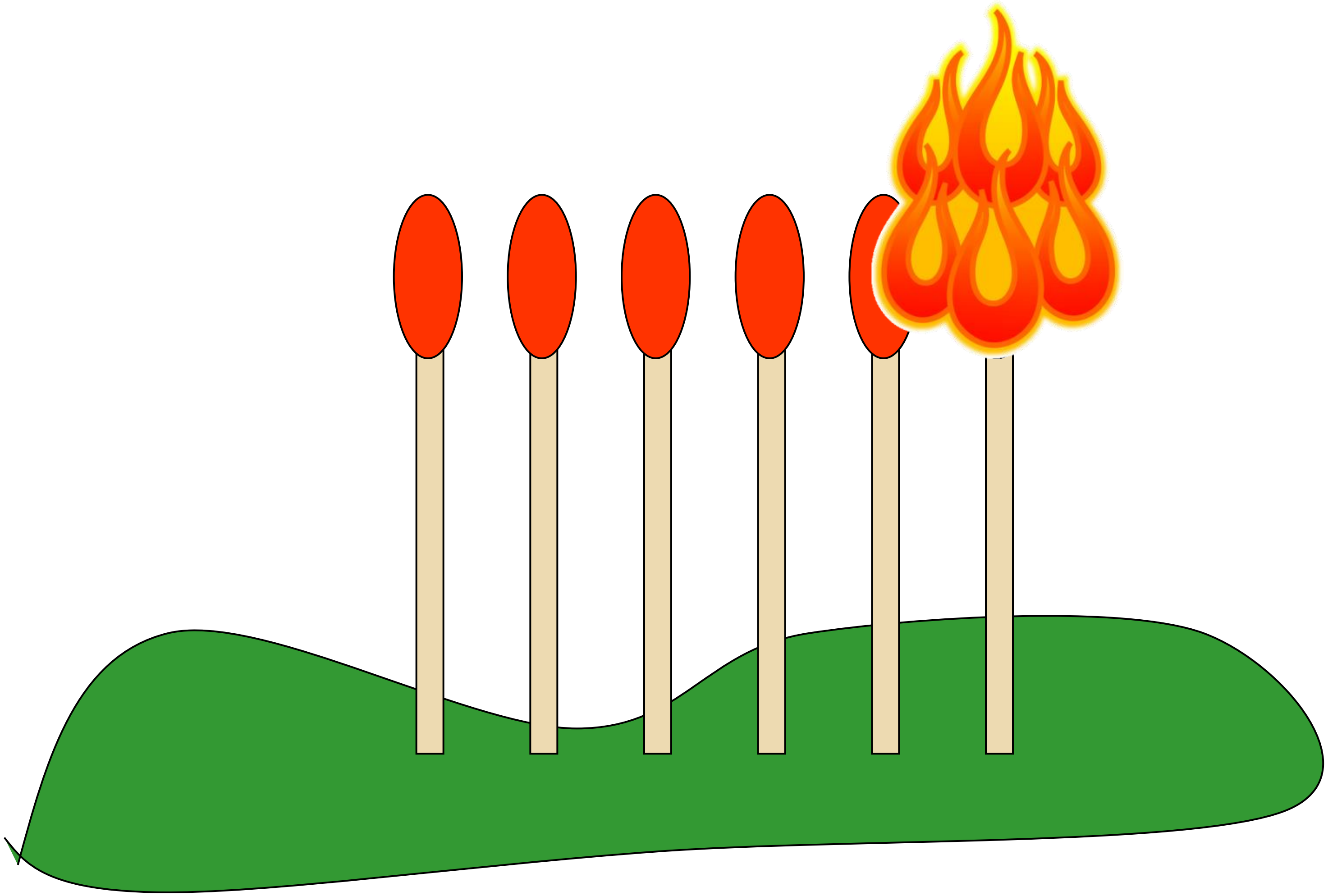

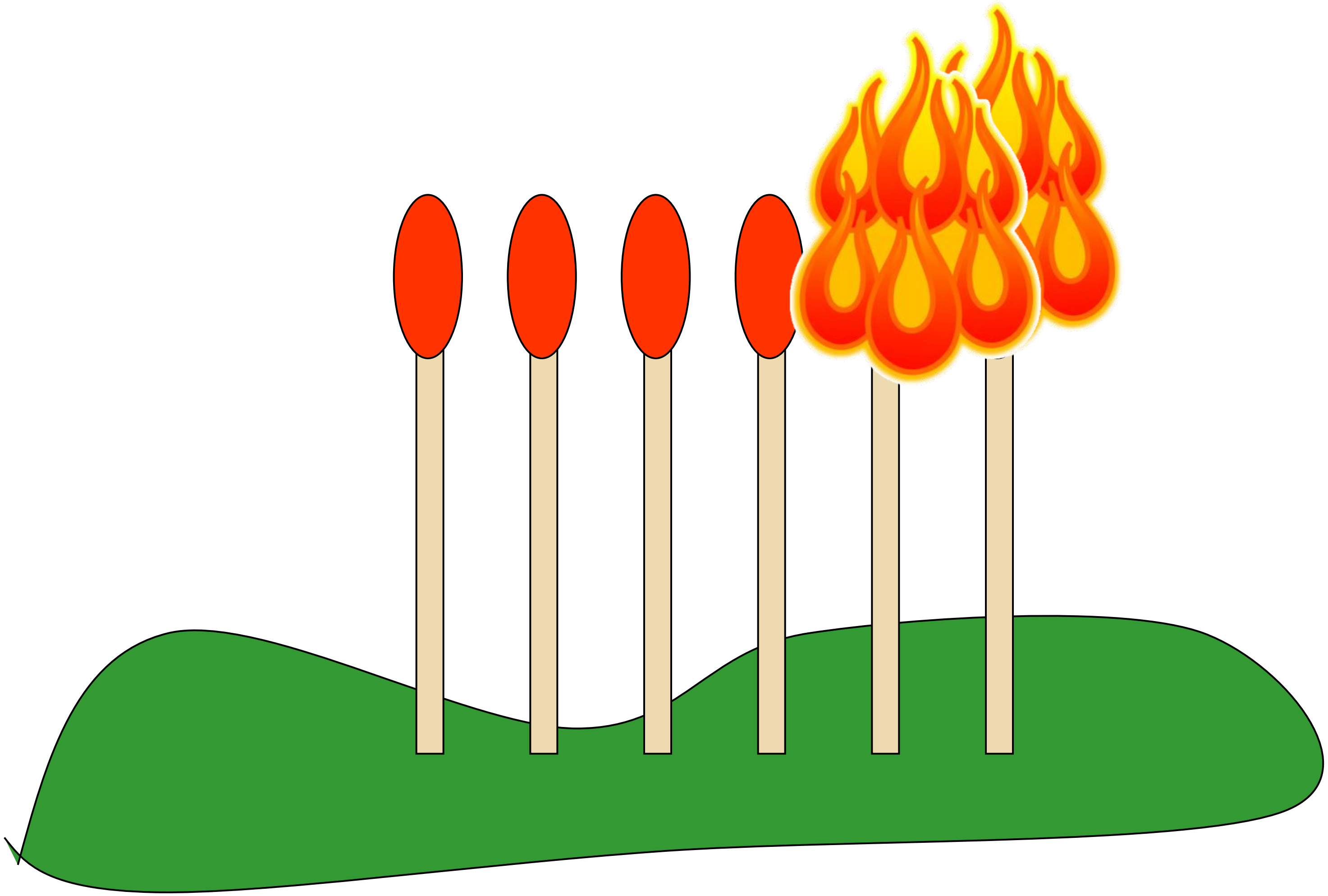

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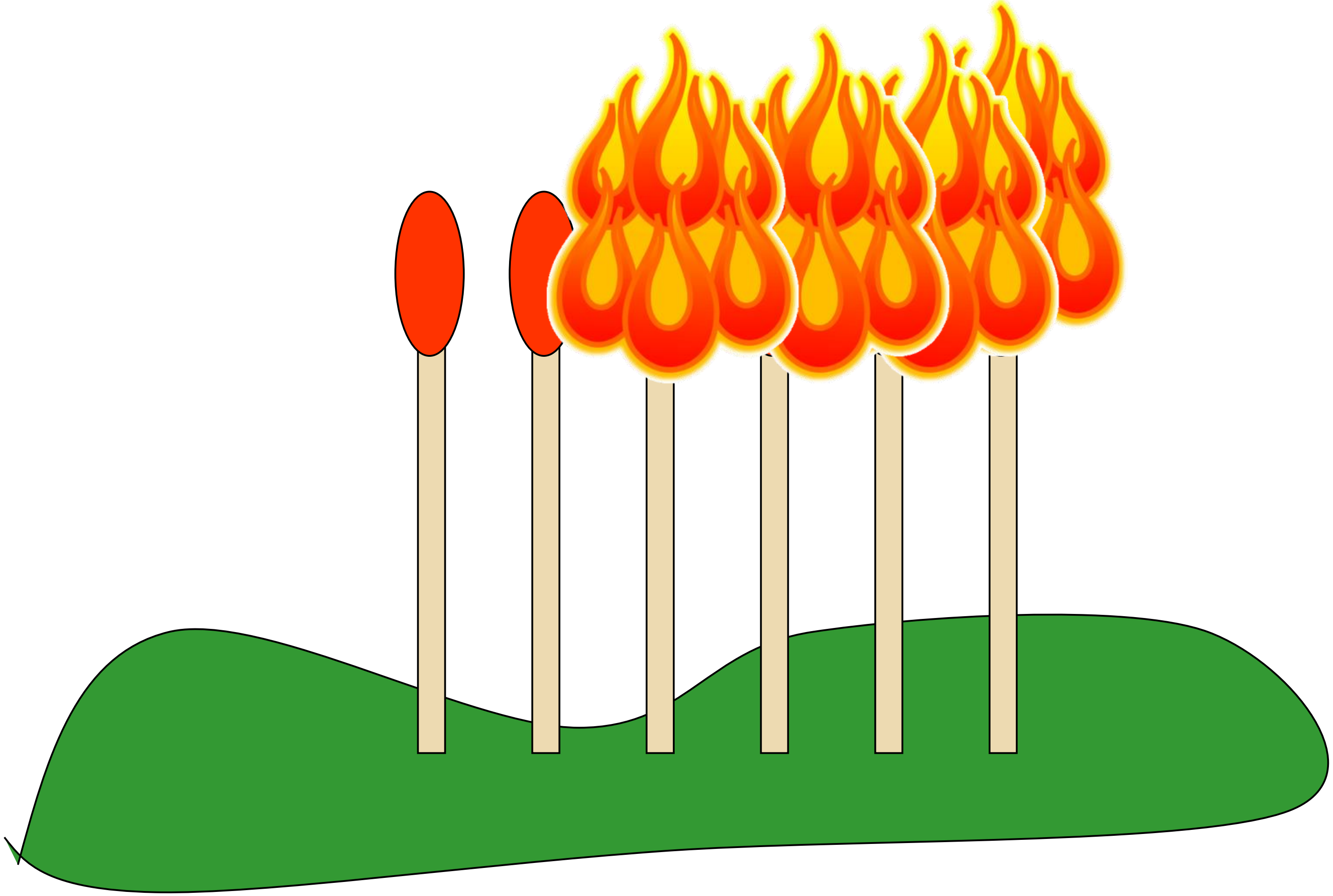

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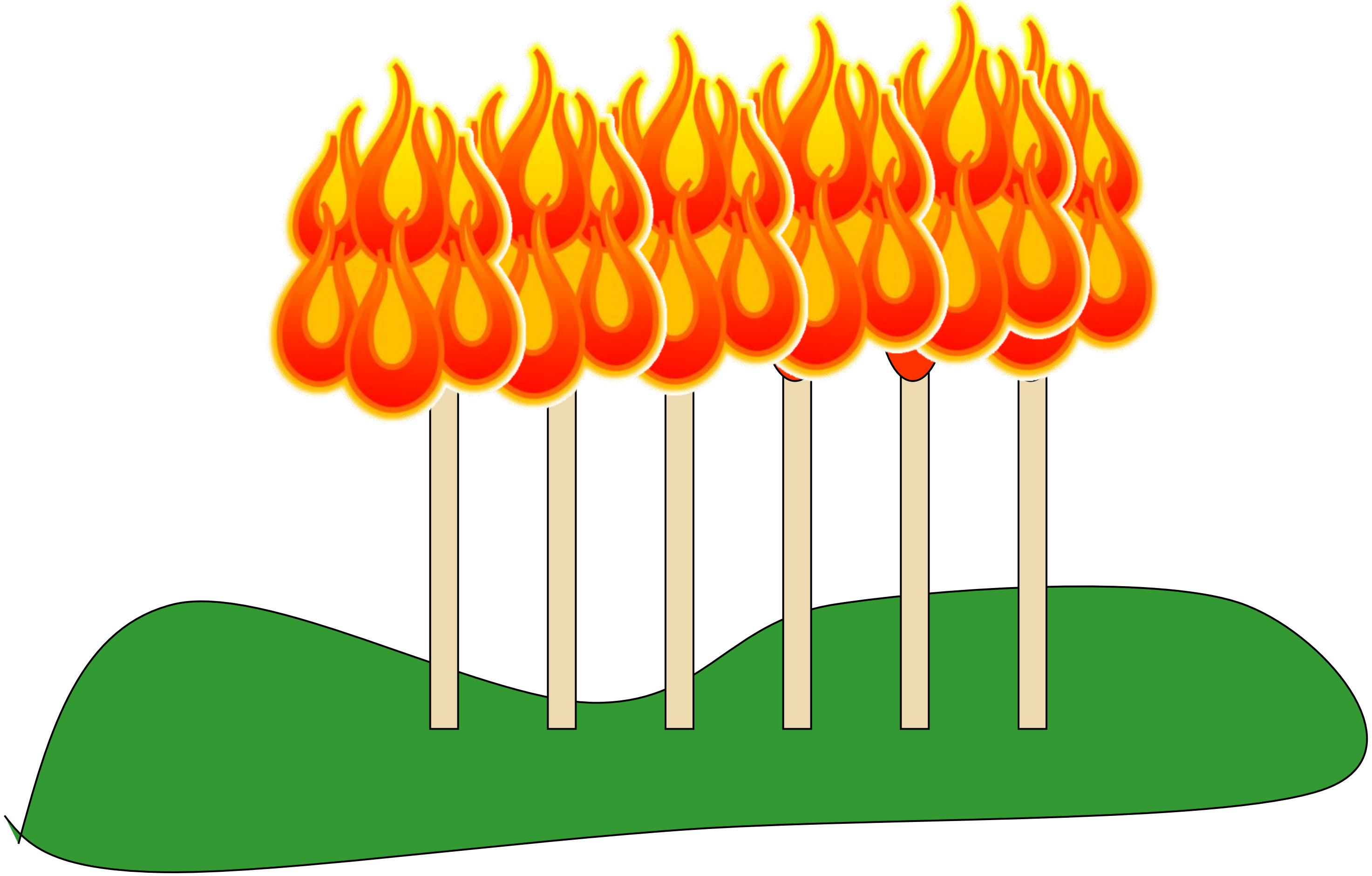
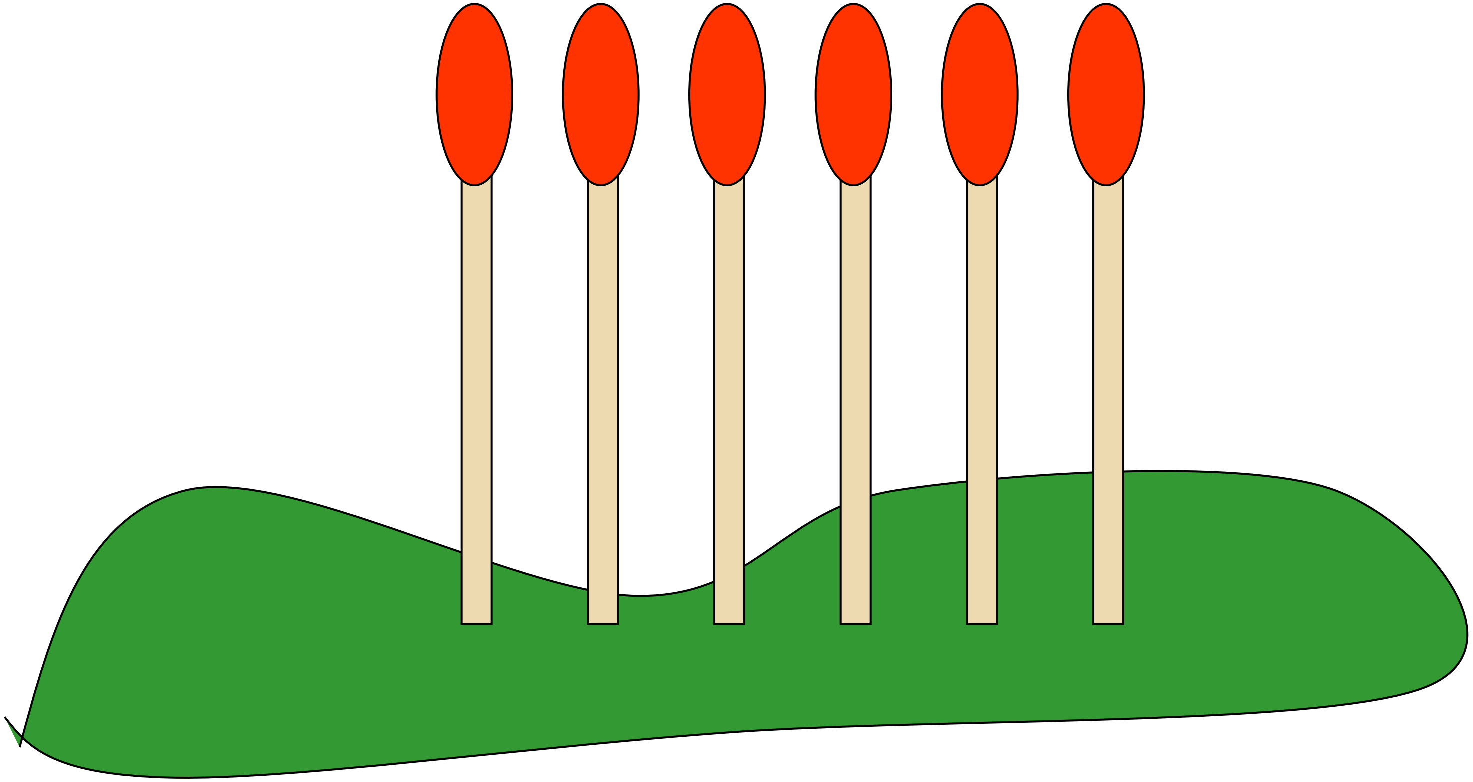


SOLUTION?



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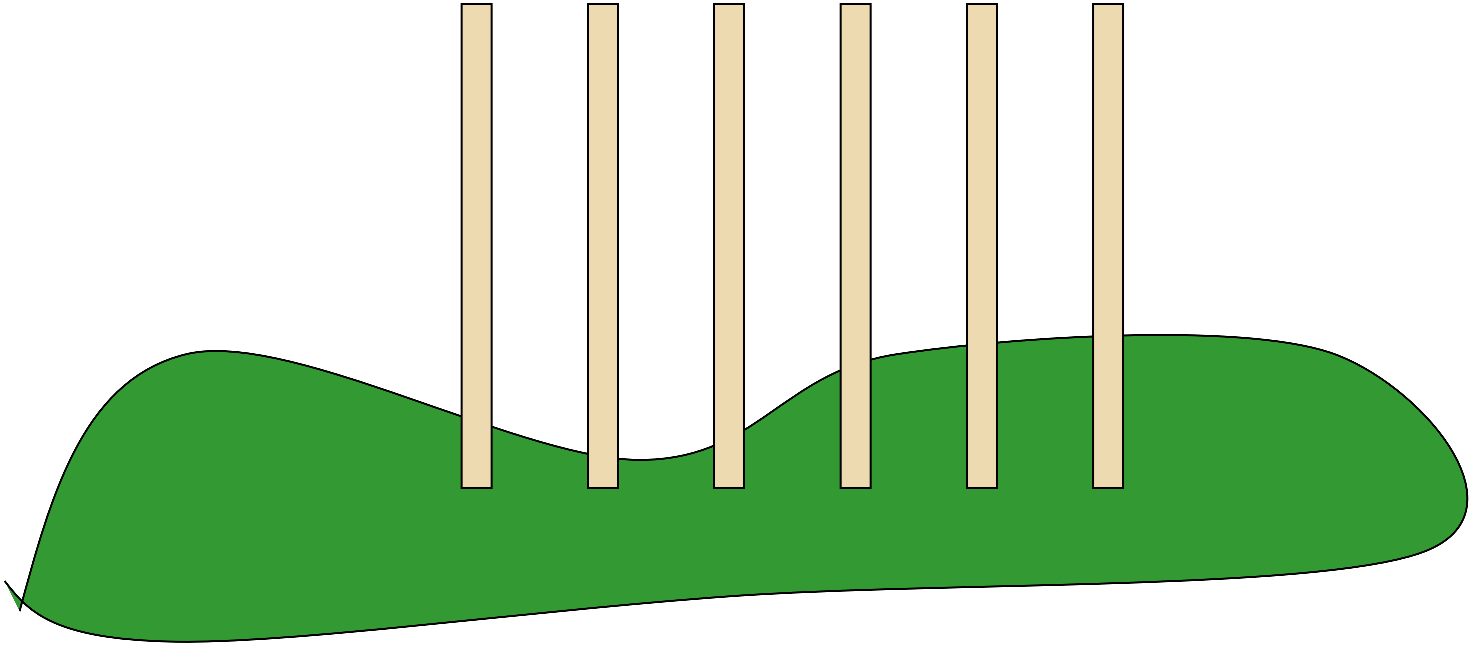
SOLUTION?



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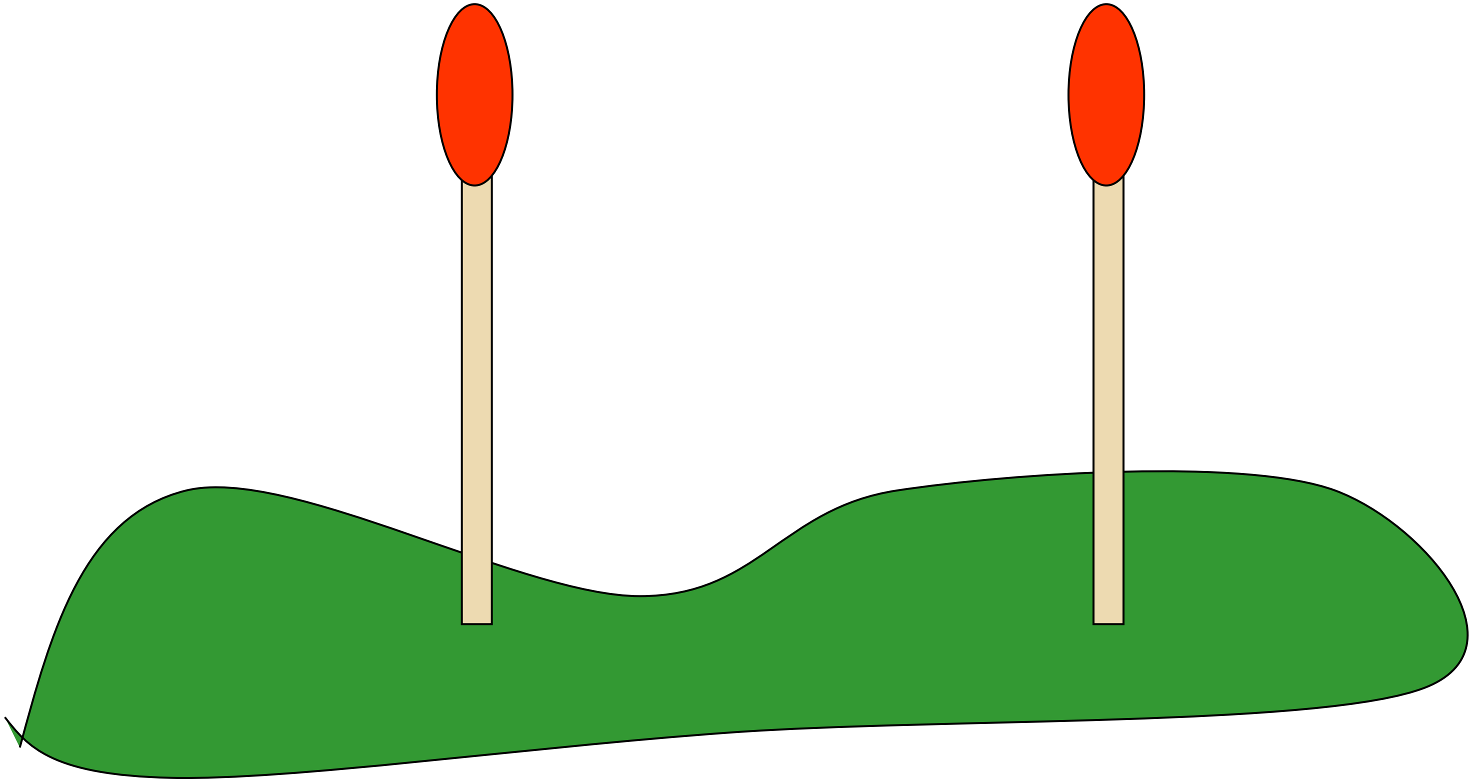
SOLUTION?



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In the Pond of Toxicology

Are there any Neon Swans?

Food, Air, Water,
Chemicals,
Drugs,,,,,

Coordinated Basic and Applied Science should make us foresee and see the swans.

End

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