H. 知的財産権の出願・登録状況

1．特許取得

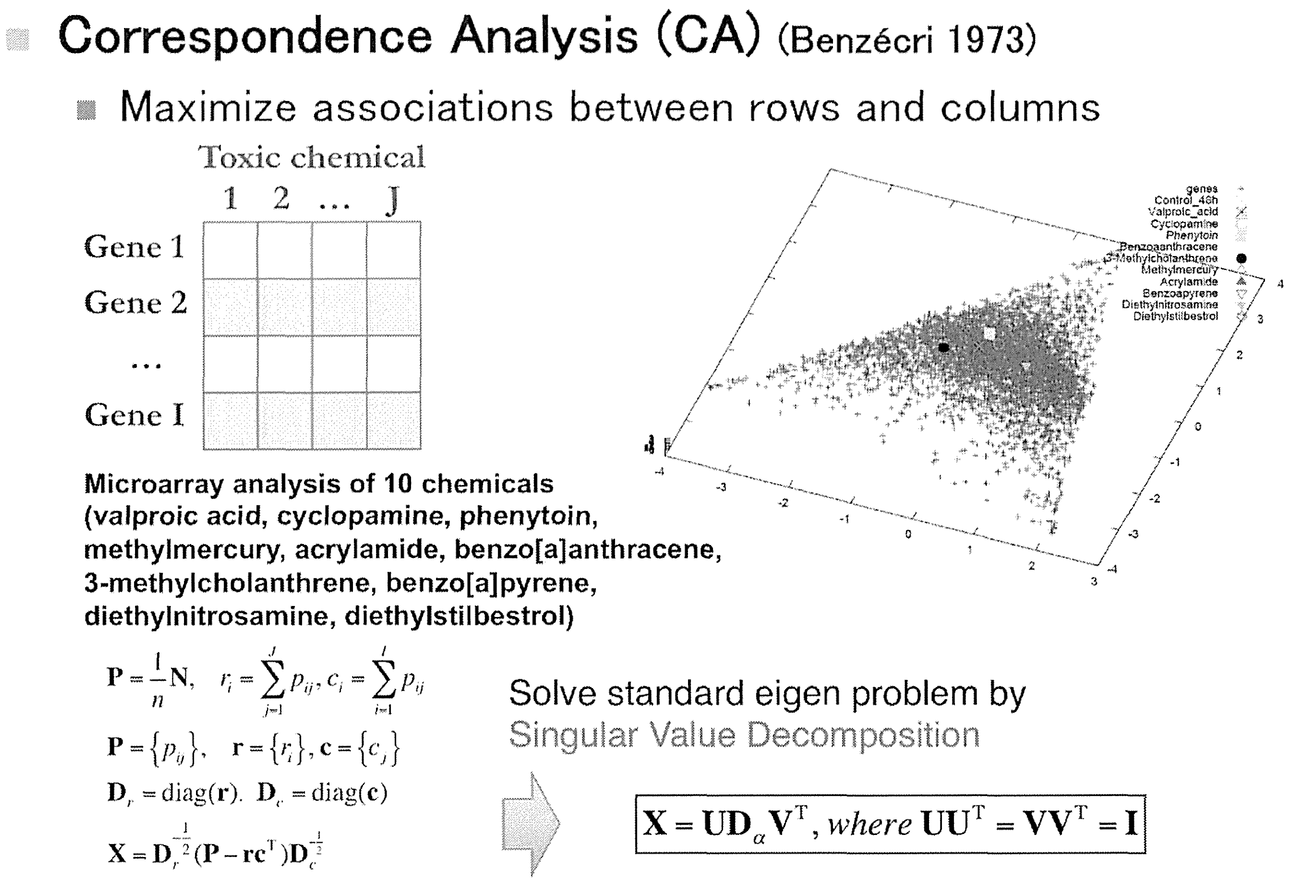
無し

2．実用新案登録

無し

3．その他

無し



Correspondence Analysis (CA) (Benzécri 1973)

- Maximize associations between rows and columns

Microarray analysis of 10 chemicals (valproic acid, cyclopamine, phenytoin, methylmercury, acrylamide, benzo[a]anthracene, 3-methylcholanthrene, benzo[a]pyrene, diethylnitrosamine, diethylstilbestrol)

$$\mathbf{P} = \frac{1}{n}\mathbf{N}, \quad r_i = \sum_{j=1}^{J} p_{ij}, c_i = \sum_{i=1}^{I} p_{ij}$$

$$\mathbf{P} = \{p_{ij}\}, \quad \mathbf{r} = \{r_i\}, \mathbf{c} = \{c_j\}$$

$$\mathbf{D}_r = \mathrm{diag}(\mathbf{r}), \ \mathbf{D}_c = \mathrm{diag}(\mathbf{c})$$

$$\mathbf{X} = \mathbf{D}_r^{-\frac{1}{2}}(\mathbf{P} - \mathbf{r}\mathbf{c}^{\mathrm{T}})\mathbf{D}_c^{-\frac{1}{2}}$$

Solve standard eigen problem by Singular Value Decomposition

$$\boxed{\mathbf{X} = \mathbf{U}\mathbf{D}_\alpha\mathbf{V}^{\mathrm{T}}, \textit{where } \mathbf{U}\mathbf{U}^{\mathrm{T}} = \mathbf{V}\mathbf{V}^{\mathrm{T}} = \mathbf{I}}$$

図１：**Correspondence Analysis（CA）と毒性化合物に対応する遺伝子の三次元展開**

ES 細胞株(KhES3)に対して 10 化合物及び 1 コントロールを使用して 11 の固有値に展開し、そこから生物学的な基準をクリアする遺伝子 10 個を選択した。

Gene Symbol	**Component (Weight)**	**Definition***	**Synonym**
NANOG	5 (3.03)	*Homo sapiens* Nanog homeobox (NANOG), mRNA.	–
SOX2	1 (1.64), 4 (2.19)	*Homo sapiens* SRY (sex determining region Y)-box 2 (SOX2), mRNA.	ANOP3; MGC2413; MCOPS3
DMTF1	6 (−2.44)	*Homo sapiens* cyclin D binding myb-like transcription factor 1 (DMTF1), mRNA.	DMP1; DMTF; hDMP1; FLJ41265
ZNF208	6 (−2.54), 10 (−3.71)	*Homo sapiens* zinc finger protein 208 (ZNF208), mRNA.	ZNF95; PMIDP
ADRM1	1 (1.70)	*Homo sapiens* adhesion regulating molecule 1 (ADRM1), transcript variant 2, mRNA.	Rpn13; GP110; MGC29536
TRIB1	1 (−3.25), 2 (−2.77)	*Homo sapiens* tribbles homolog 1 (*Drosophila*) (TRIB1), mRNA.	GIG2; SKIP1; C8FW
CRY1	4 (2.47)	*Homo sapiens* cryptochrome 1 (photolyase-like) (CRY1), mRNA.	PHLL1
SMAD7	1 (1.66)	*Homo sapiens* SMAD family member 7 (SMAD7), mRNA.	FLJ16482; MADH8; MADH7
SMAD6	6 (3.68), 7 (−2.88), 8 (2.72), 9 (−2.49)	*Homo sapiens* SMAD family member 6 (SMAD6), transcript variant 1, mRNA.	MADH6; HsT17432; MADH7
VHL1	1(1.77), 2 (−2.72), 4 (2.49)	*Homo sapiens* von Hippel-Lindau tumor suppressor (VHL), transcript variant 2, mRNA.	HRCA1; RCA1; VHL1

図２：Correspondence Analysis と４つの生物学的基準で抜き出した遺伝子のリスト

*Definition には illumina 社マイクロアレイに付属しているアノテーションをそのまま引用した。

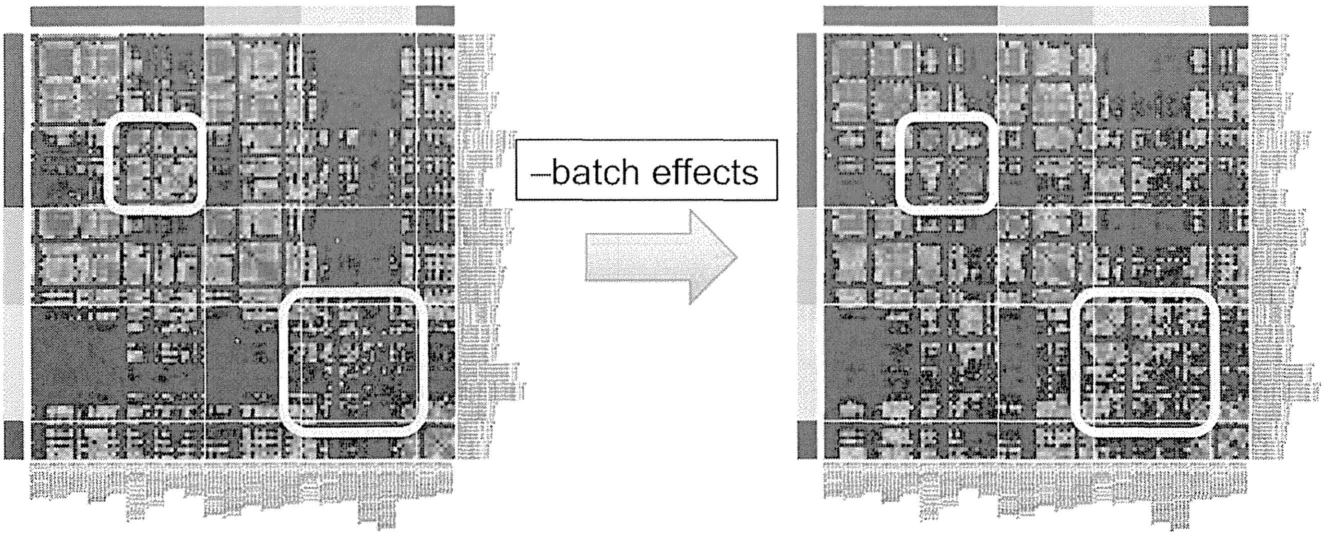
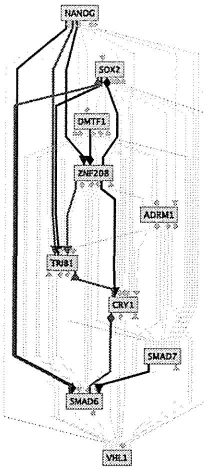


図3：経験的ベイズ法による線形モデリングを利用したノイズ除去

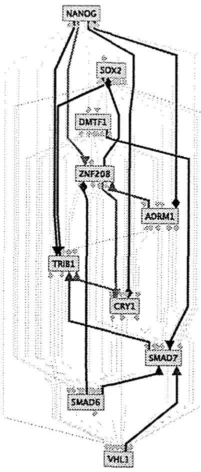
96 well x 10 プレートを用いた qRT-PCR 毒性試験データから 22 化合物間でのピアソン相関係数。（左）ノイズ除去前には、バッチ効果ノイズによる遺伝子発現データの意味のない相関が得られているが、（右）ノイズ除去後にはこれが軽減され、より小さな化合物単位での類似遺伝子発現データが増えていることと（左上の枠内）、同じ毒性グループでの相関が増している（右下の枠内）。

Neurotoxins

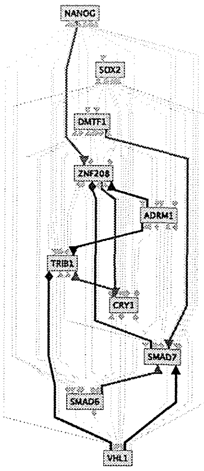
Valproic acid



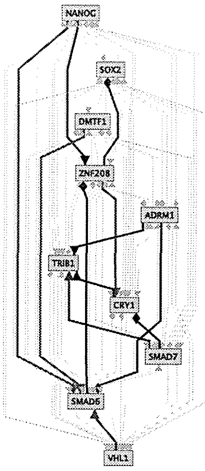
Cyclopamine



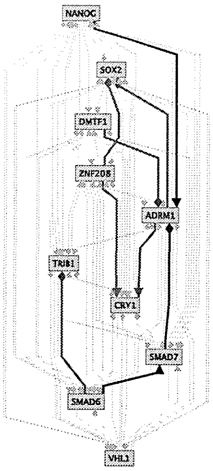
Phenytoin



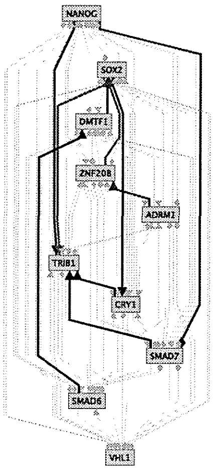
Methylmercury



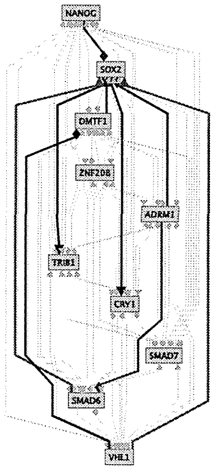
Acrylamide



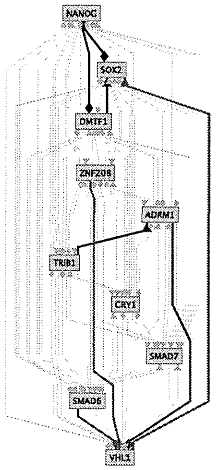
4-OH-2',3,3',4',5'-PCB



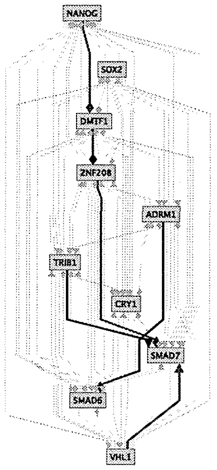
2,5-Hexanedione



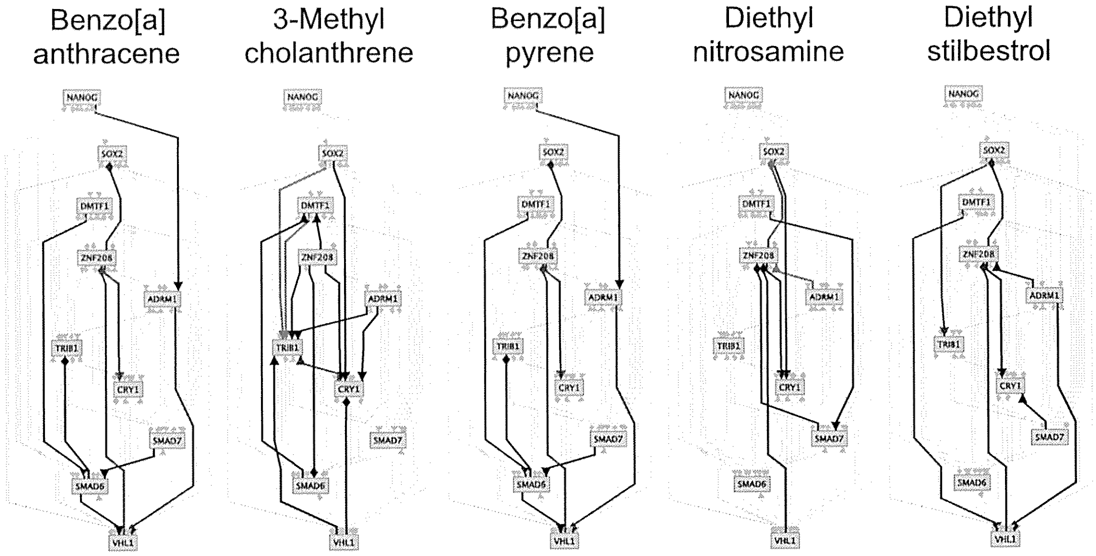
Warfarin



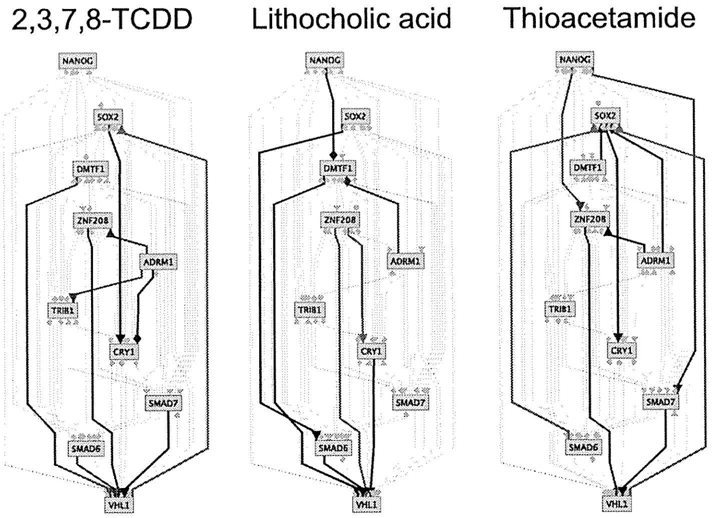
Thalidomide



Genotoxic carcinogens



Non-genotoxic carcinogens



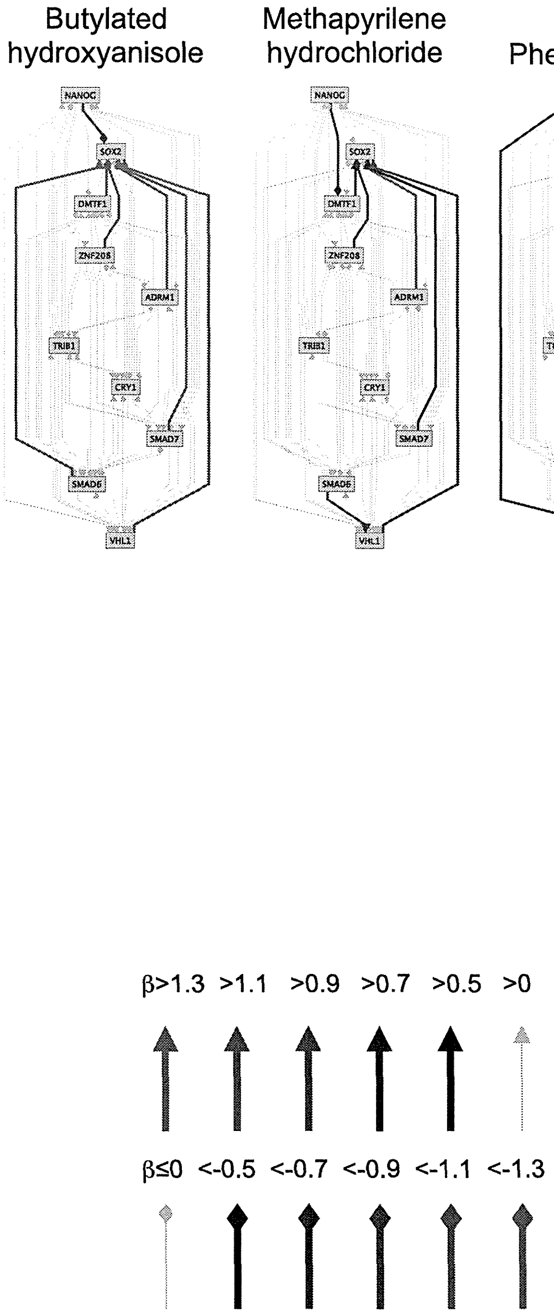


図4：ベイジアンネットワーク推定による 20 化合物の遺伝子ネットワーク

10 遺伝子の配置を同じにして、神経毒性(9)、遺伝的発がん性(5)、非遺伝的発がん性(6)のそれぞれの毒性カテゴリーについてネットワークを描いた結果。同じカテゴリー内でも共通性が見つかりにくいことはカテゴリー内でのサブカテゴリーがあることを示唆している。

Compound Name	Dose (nM)						Toxicity Category
Valproic acid	1000	500	250	125	62.5	0	Neurotoxin (NT)
Cyclopamine	1000	500	250	125	62.5	0	
Phenytoin	1000	500	250	125	62.5	0	
Methylmercury	50	25	12.5	6.25	3.125	0	
Acrylamide	5000	2500	1250	625	312.5	0	
4-OH-2',3,3',4',5'-PCB107	10	5	2.5	1.25	0.625	0	
2,5-Hexanedione	1000	500	250	125	62.5	0	
Warfarin	1000	500	250	125	62.5	0	
Thalidomide	500	250	125	62.5	31.25	0	
Benzo[a]anthracene	50	25	12.5	6.25	3.125	0	Genotoxic carcinogen (GC)
3-Methylcholanthrene	500	250	125	62.5	31.25	0	
Benzo[a]pyrene	500	250	125	62.5	31.25	0	
Diethylnitrosamine	500	250	125	62.5	31.25	0	
Diethylstilbestrol	500	250	125	62.5	31.25	0	
2,3,7,8-TCDD	10	5	2.5	1.25	0.625	0	Non-genotoxic carcinogen (NGC)
Lithocholic acid	1000	500	250	125	62.5	0	
Thioacetamide	1000	500	250	125	62.5	0	
Butylated hydroxyanisole	1000	500	250	125	62.5	0	
Methapyrilene hydrochloride	1000	500	250	125	62.5	0	
Phenobarbital	1000	500	250	125	62.5	0	
Bisphenol-A	1000	500	250	125	62.5	0	Uncategorized
Permethrin	1000	500	250	125	62.5	0	

図5：今回の測定に用いた 20 毒性既知化合物＋2 未知化合物及びその曝露濃度

上から神経毒性(9)、遺伝的発がん性(5)、非遺伝的発がん性(6)に分類している。

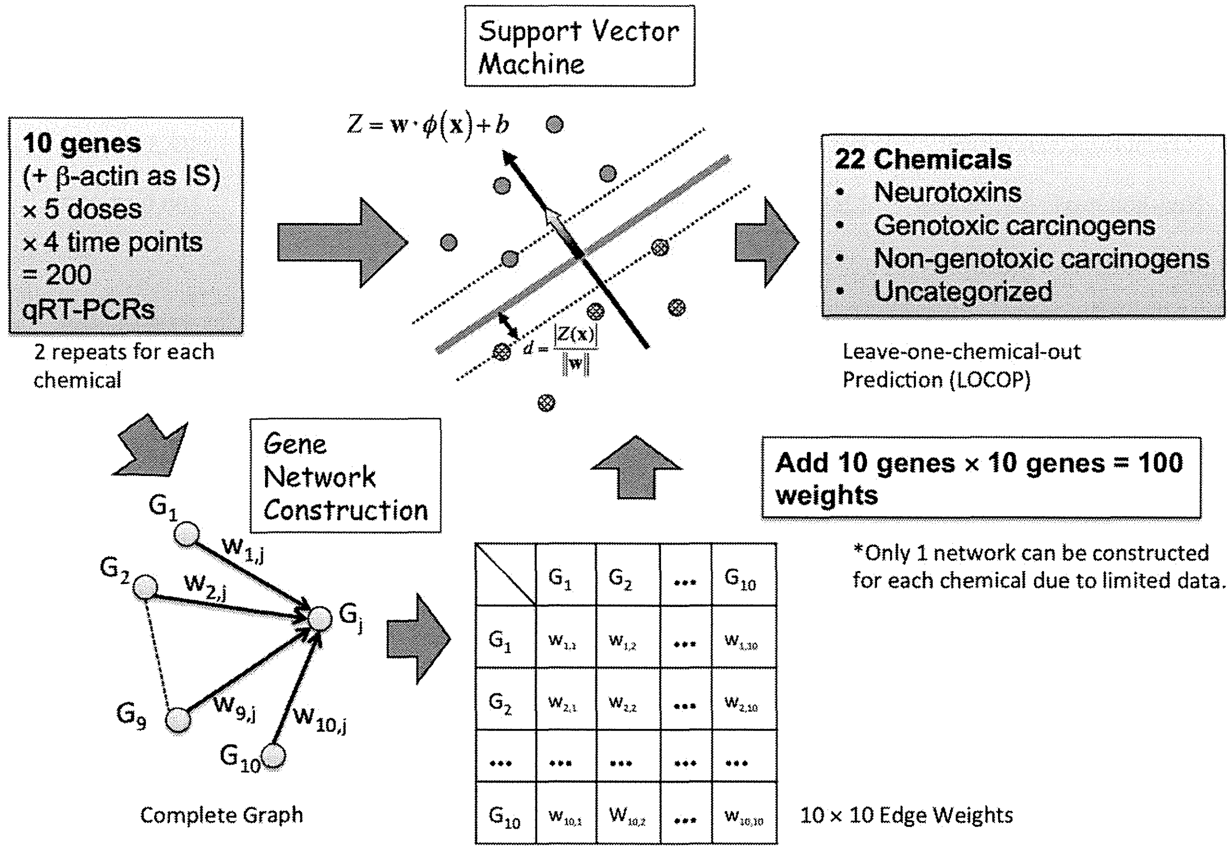
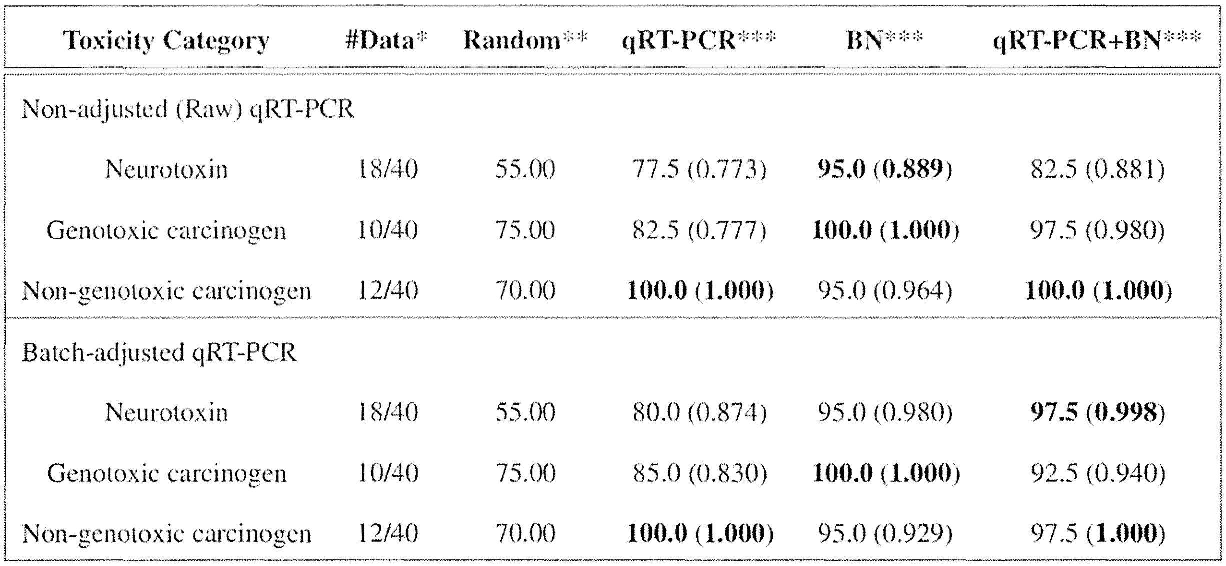


図6：予測の全体像

10遺伝子+1外部標準遺伝子を出発として遺伝子ネットワークを予測し、そのエッジをSVMに加えて予測した。



Toxicity Category	**#Data***	**Random****	**qRT-PCR*****	**BN*****	**qRT-PCR+BN*****
Non-adjusted (Raw) qRT-PCR					
Neurotoxin	18/40	55.00	77.5 (0.773)	**95.0 (0.889)**	82.5 (0.881)
Genotoxic carcinogen	10/40	75.00	82.5 (0.777)	**100.0 (1.000)**	97.5 (0.980)
Non-genotoxic carcinogen	12/40	70.00	**100.0 (1.000)**	95.0 (0.964)	**100.0 (1.000)**
Batch-adjusted qRT-PCR					
Neurotoxin	18/40	55.00	80.0 (0.874)	95.0 (0.980)	**97.5 (0.998)**
Genotoxic carcinogen	10/40	75.00	85.0 (0.830)	**100.0 (1.000)**	92.5 (0.940)
Non-genotoxic carcinogen	12/40	70.00	**100.0 (1.000)**	95.0 (0.929)	97.5 (**1.000**)

図7：SVMによる毒性カテゴリー予測結果

左から、毒性カテゴリー、データ数、ランダムな場合の予測率、qRT-PCRのみの予測率、ベイジアンネットワークのみの予測率、qRT-PCR+ベイジアンネットワークでの予測率。

研究成果の刊行一覧表

別紙４

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