

R2 = 0.943879

変数	DF	Type I SS	Mean Square	F 値	Pr > F
EXPR	3	6487.933500	2162.644500	66.15	<.0001
TRT	4	110.602000	27.650500	0.85	0.5227

EXPR

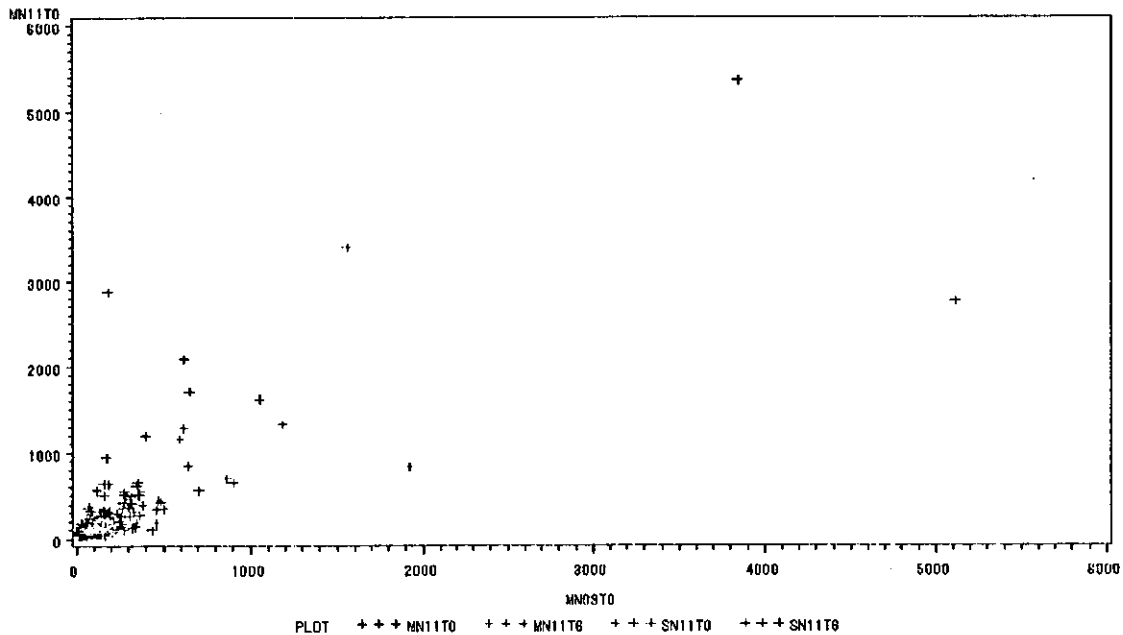
MCF7, E11	65.04
MCF7, E9	50.10
SK-BR-3, E11	49.44
SK-BR-3, E9	15.84

TRT

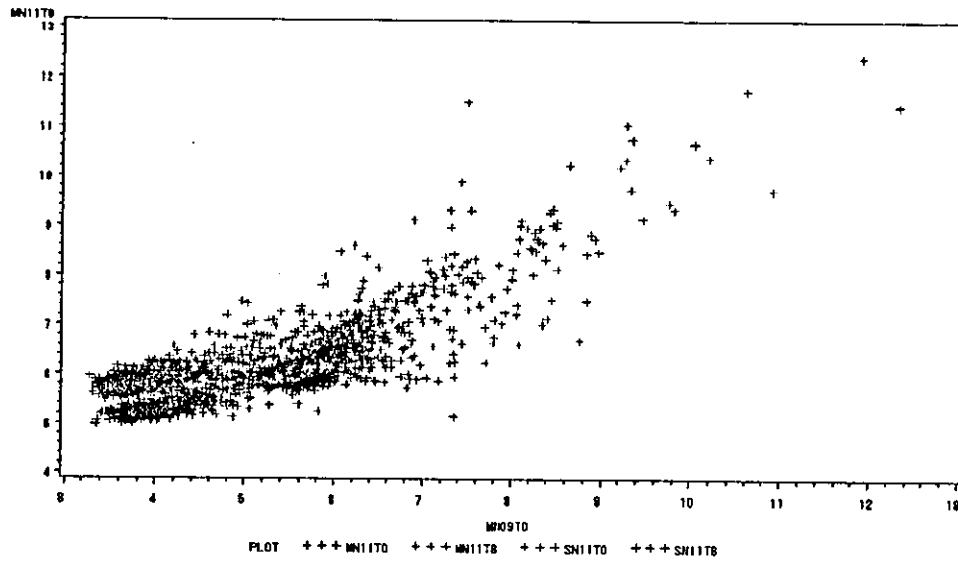
Cont.	47.750
E	42.900
E+T7	47.500
E+T6	45.425
T6	41.950

2. 散布図

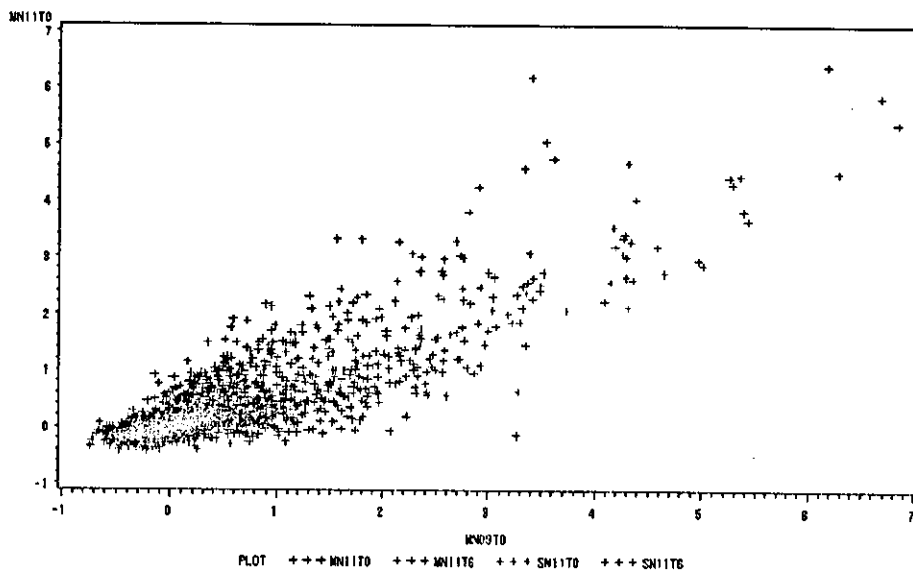
I. 同一処理の細胞間



II. 散布図 (log2 変換)

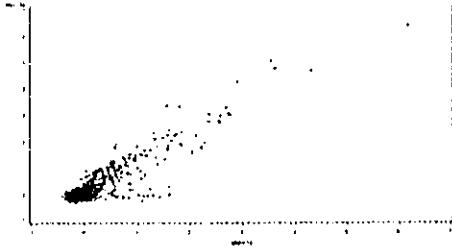


III. 散布図 (中央値による正規化)

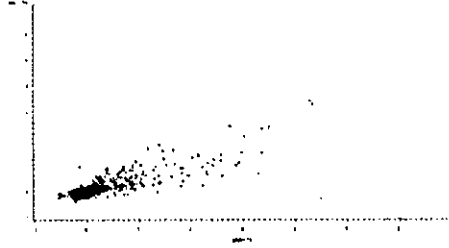


IV. 散布図 (同一処理の細胞毎)

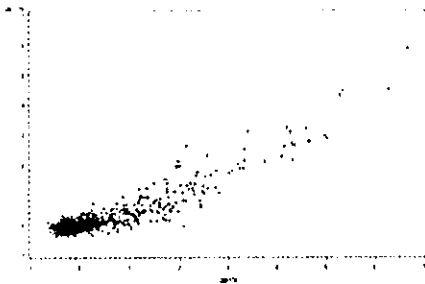
MN**T0



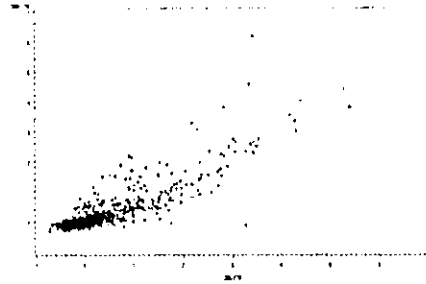
MN**T6



SN**T0



SN**T6

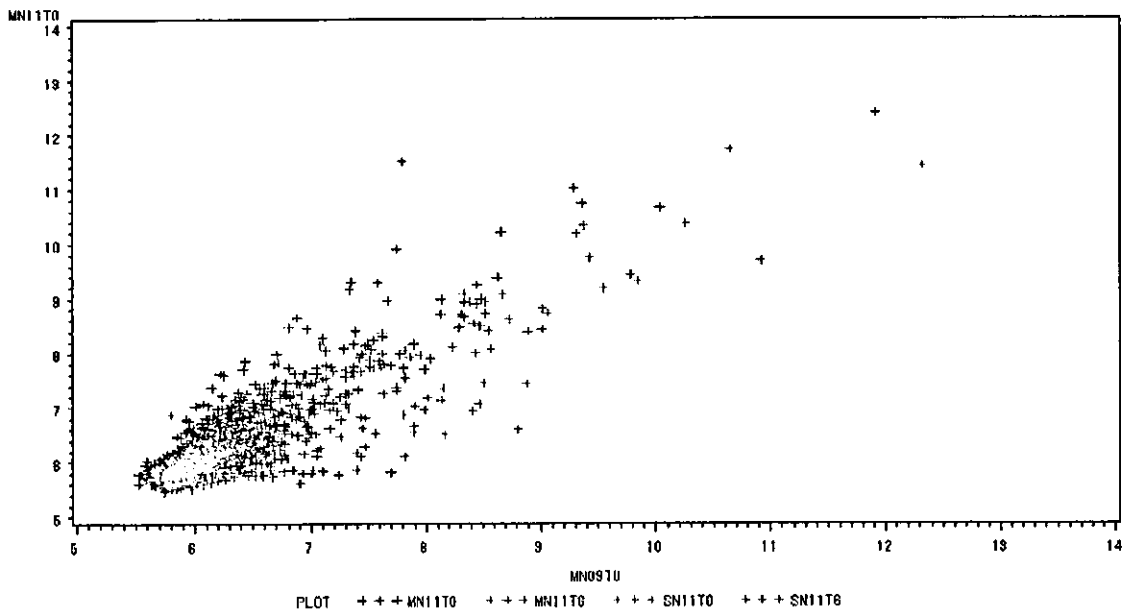


考察

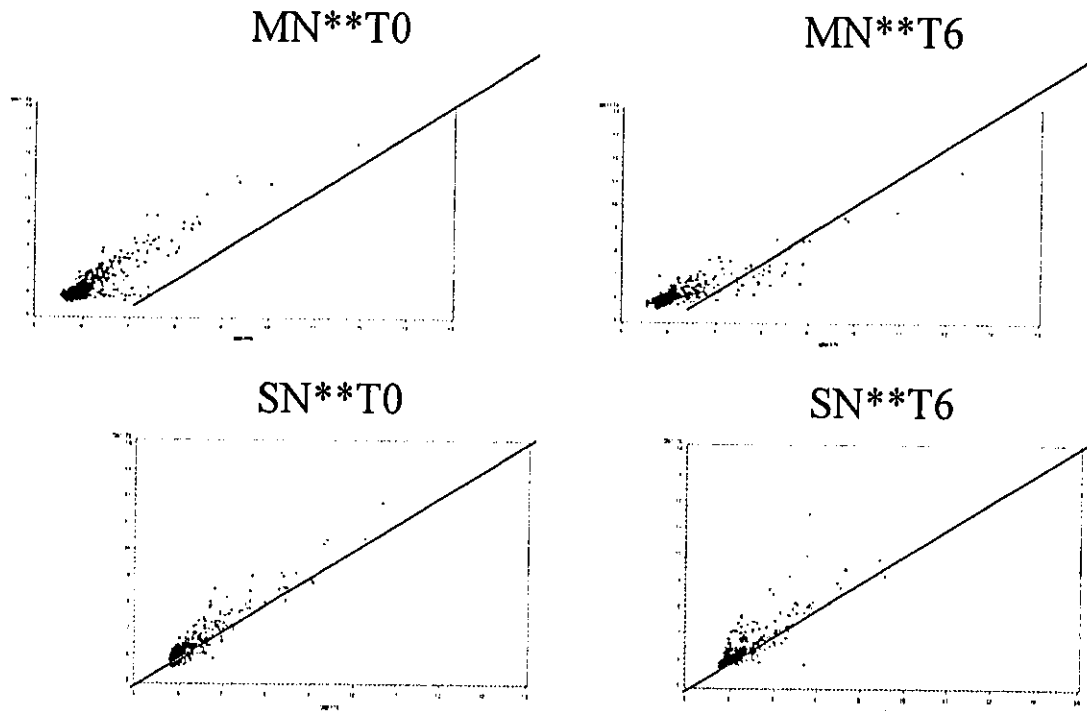
- 正規化後に非線形の傾向がみられる
- 本来は 45 度の直線に従って分布する
- 非線形の度合は散布図毎に異なる
- 平均値や中央値の乖離が大きいものほど、非線形の度合が大きい傾向がある

2. 正規化

I. 対数変換前に正規化した場合



II. 散布図 (同一処理の細胞毎)

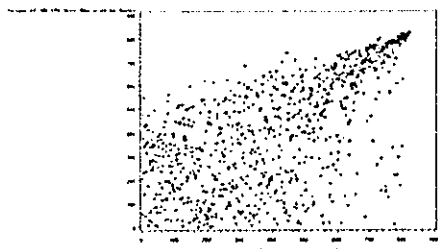


考察

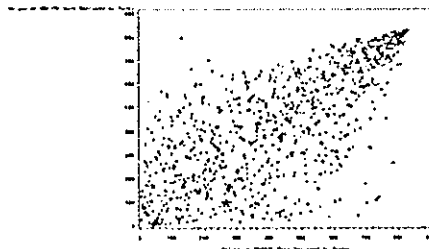
- 対数変換に由来するバイアスは 正規化→(比)→対数変換の順が良好な結果を得られる。
- 順位変換は対数変換や正規化の影響を受けない
- 実験変動に対してロバスト

III. 散布図 (順位変換)

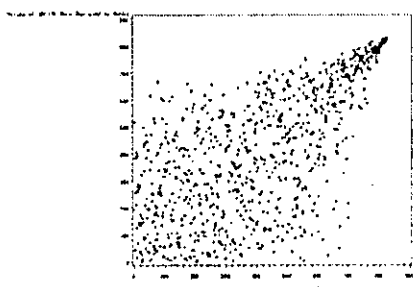
MN**T0



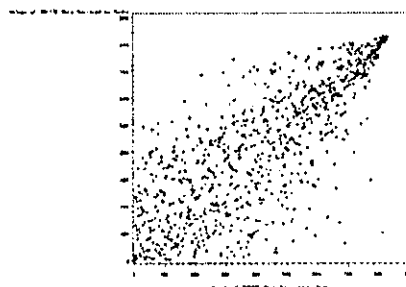
MN**T6



SN**T0

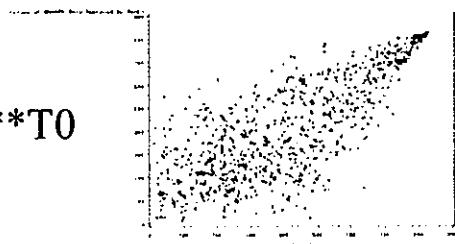


SN**T6

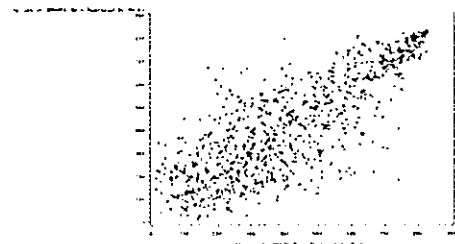


IV 順位の平均をとった散布図

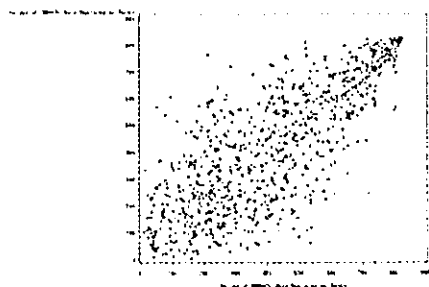
MN**T6



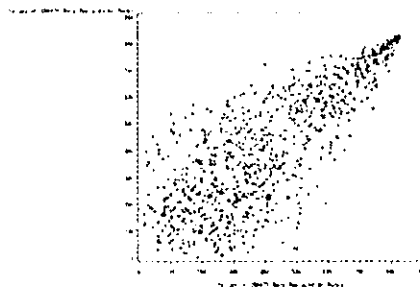
SN**T0



MN**T0

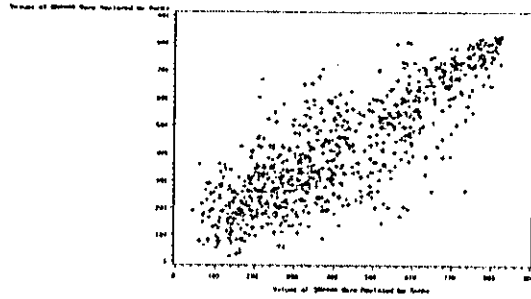


SN**T6

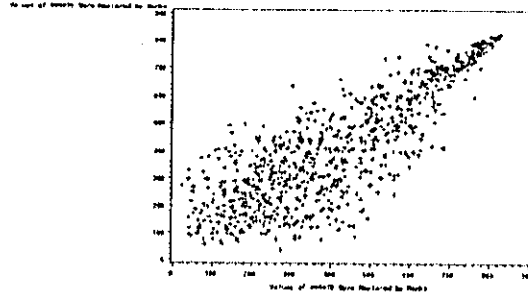


V. 順位の平均をとった散布図

MN vs SN

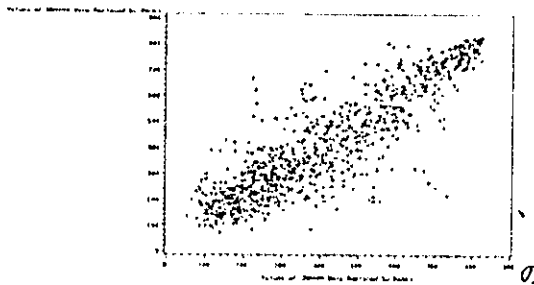


T0 vs T6

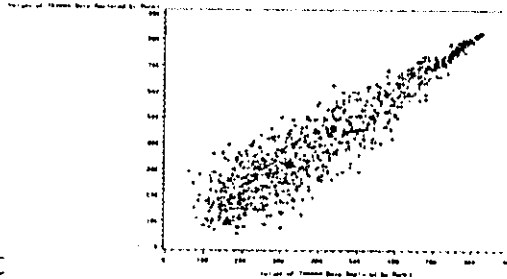


VI. 順位の平均をとった散布図

MN vs SN



T0 vs T6



3. クラスター解析

I. 全遺伝子の Centroid linkage

順位データを下記のように変換

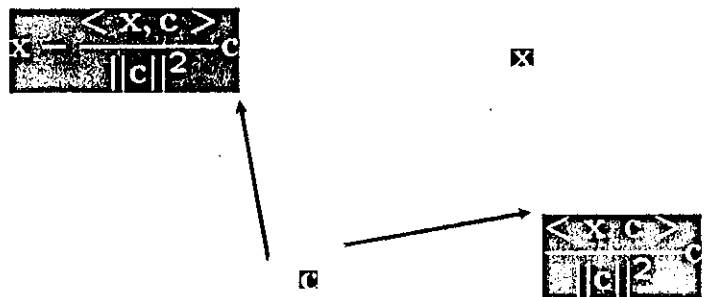
$$y = 2\{2(x-1)/(n-1)-1\}$$

遺伝子に対して Centroid linkage を行った

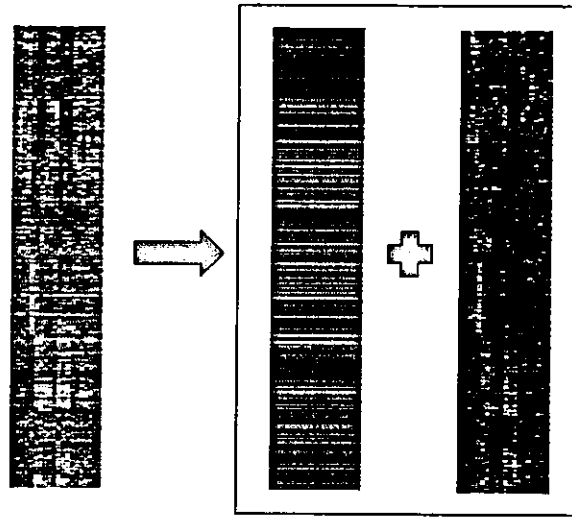


II. アレイデータの分解

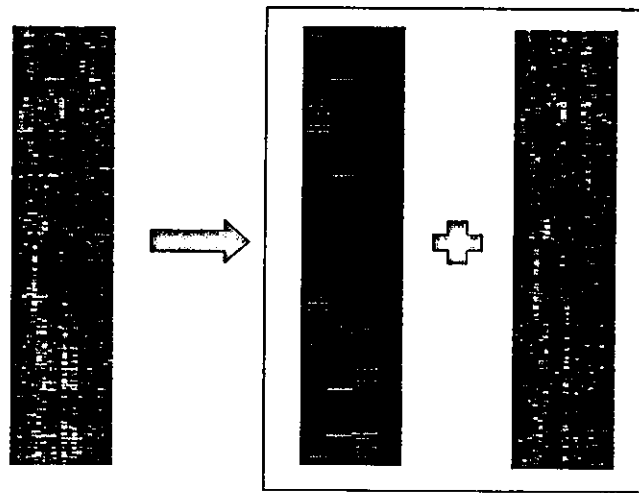
- アレイデータを対比空間に正射影
- 直交するふたつのベクトルに分解



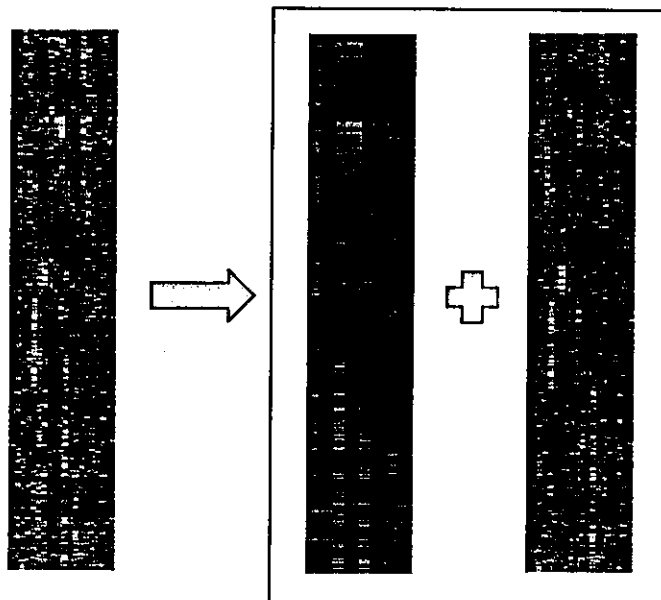
アレイデータの分解 (1)



アレイデータの分解 (2)



アレイデータの分解 (3)



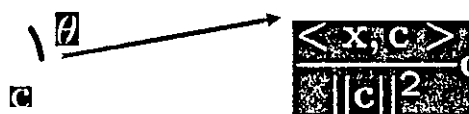
考察

- アレイデータの分解は、視覚的にデータの特徴を吟味する際に有用である。
- ただし、偶然生じたノイズをシグナルとして誤って拾う可能性も高い。
- シグナルの順位付けが必要。

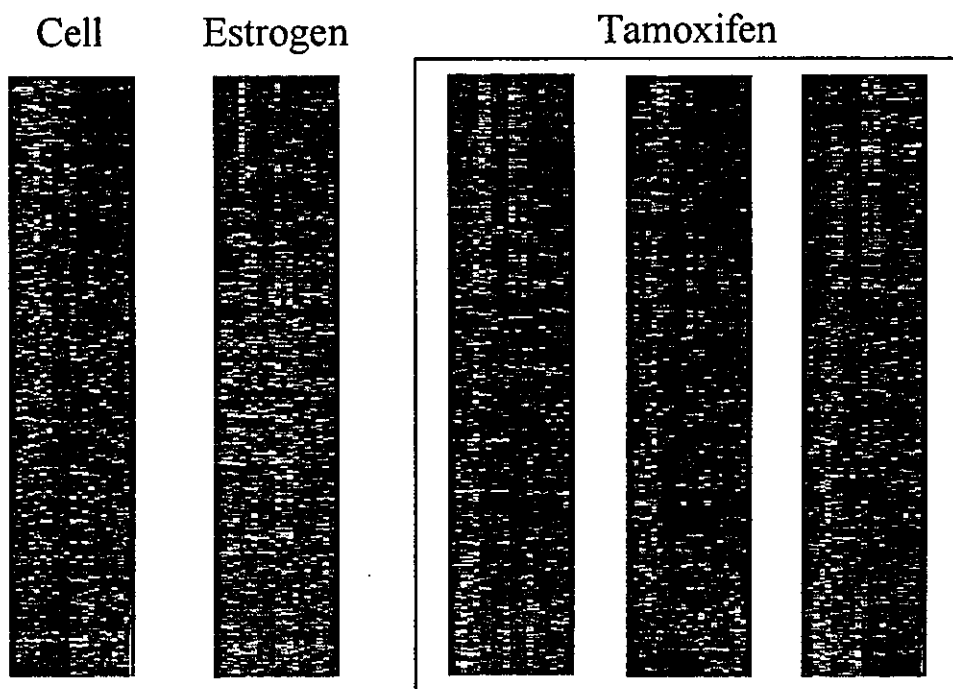
4. ベクトル間の角度による順位付け

$$\cos\theta = \frac{\langle x, c \rangle}{\|x\| \|c\|}$$

⊗



I. 特異的な発現変動の抽出



II. 細胞株間による発現遺伝子の抽出

乳癌細胞株 I に強く発現している遺伝子群

Cytokine; interleukin 18 precursor (IL18); interferon gamma-inducing factor (IFN-gamma-inducing factor; IGIF); interleukin 1 gamma (IL1-gamma)
additional; insulin receptor substrate 1
signal/oncogene; retinoid X receptor alpha (RXRA)
translation/detoxification; glutathione-S-transferase homolog
cell cycle regulation; cyclin-dependent kinase 4 inhibitor 2B (CDKN2B); p14-INK4B; multiple tumor suppressor 2 (MTS2)
transporter; type I cytoskeletal 18 keratin (KRT18); cytokeratin 18 (CK18)
Transcription/Repair; high mobility group protein HMG2
signal/oncogene; jagged homolog 2 (JAG2)
signal/oncogene; microtubule-associated protein 2 (MAP2)
Drug Metabolism; nm23-H4; nucleoside-diphosphate kinase; nucleoside 5'-diphosphate phosphotransferase (NDK)
Cytokine; MHC class I molecule; MHC class I subunit-related protein A (MICA); PERB11.1
Transcription/Repair; ubiquitin-conjugating enzyme E2 17-kDa (UBE2A); ubiquitin-protein ligase; ubiquitin carrier protein; HR6A
signal/oncogene; RIBOSOMAL PROTEIN S6 KINASE (S6K)
Transcription/Repair; RAD51C truncated protein

乳癌細胞株 II に強く発現している遺伝子群

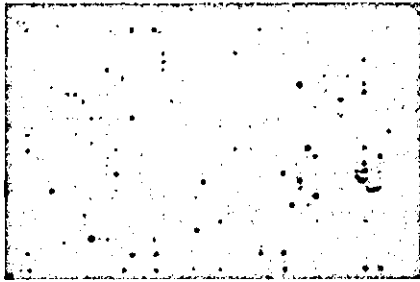
signal/oncogene; epidermal growth factor receptor (EGF receptor; EGFR); ERBB proto-oncogene
transporter; cytoskeletal keratin 7 (KRT7); cytokeratin 7 (CK7)
Cytokine; tumor necrosis factor receptor superfamily member 1A (TNFRSF1A); tumor necrosis factor receptor 1 (TNFR1); tumor necrosis factor-binding protein 1 (TBP1); CD120A antigen
signal/oncogene; ras-related protein RAB5C
transporter; type II cytoskeletal 2 oral keratin; cytokeratin 2P (CK2P)
signal/oncogene; ERBB2 proto-oncogene; NEU proto-oncogene; HER2
Transcription/Repair; thymidine phosphorylase precursor (TDRPase); platelet-derived endothelial cell growth factor (PDEC GF); gliostatin
signal/oncogene; cell surface glycoprotein MUC18; melanoma-associated antigen A32; CD146 antigen; melanoma adhesion molecule
RhoFamily; rho GDP dissociation inhibitor 2 (RHO GDI2; RHO-GDI beta); LY-GDI; ARHGDI B; GDID4
Drug Metabolism; cytochrome P450 reductase
Growth Factor; insulin-like growth factor binding protein 5 precursor (IGF-binding protein 5; IGFBP5; IBP5)
apoptosis; caspase 4 precursor (CASP4); CASP5; ICH2 cysteine protease; ICH3; TX protease; TY protease; ICE(REL) III

III. 薬剤接触により発現調節を特異的に受ける遺伝子の抽出

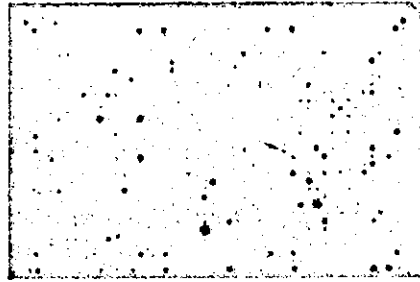
更なるノイズの低減を同法により達成し、薬剤関連遺伝子の特定を行った。結果の有意性は並べ替えにより並べ替えにより帰無分布（過程）を算出し、発現プロファイルの有意性を評価した。（非公開）

資料9 乳癌サンプルの遺伝子発現解析の実施経過

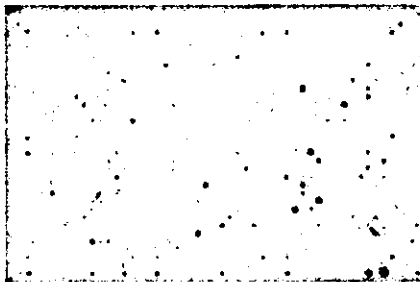
1. 現在遺伝子発現が終了した症例の提示 (症例ID は非公開とする)



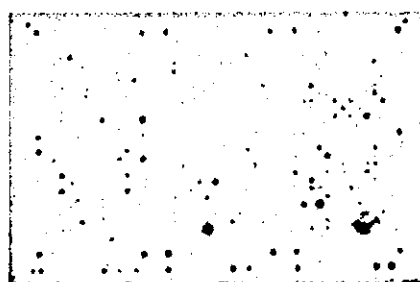
Breast cancer (Pt No.X)



PBM (Pt No.X)



Breast cancer (Pt No.X)



PBM (Pt No.X)

2. クラスタ解析結果例

乳がんと末梢血単核球の遺伝子発現プロファイルはクラスタ解析により、明確に区別された。



B:乳がん
L:末梢血単核球

3. 臨床乳癌サンプルで発現の高い遺伝子の選択 (中間解析)

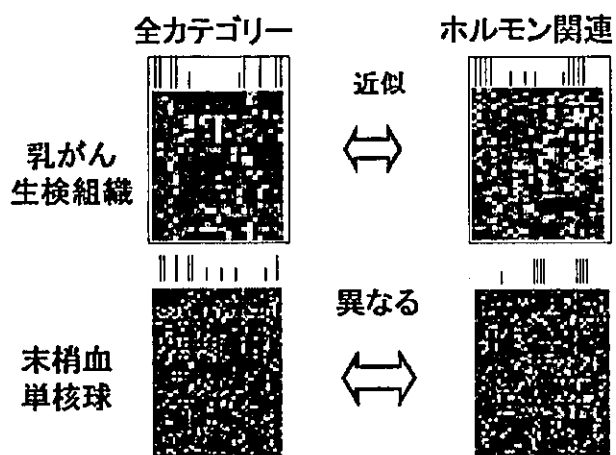
- a) 乳癌で発現の高い遺伝子(PBMC との比較)
 cadherin 3 (CDH3); placental cadherin precursor (P-cadherin: CDHP)
 proliferating cyclic nuclear antigen (PCNA); cyclin

casein kinase I gamma 3 subunit (CSNK1G3)
insulin receptor precursor (INSR; IR)
heat shock protein 40 homolog (HSP40 homolog); DNAJW
wingless-related MMTV integration site 8b protein (WNT8B)
BCL2-interacting killer protein (BIK); NBK apoptotic inducer protein; BP4; BIP1
cadherin 6 precursor (CDH6); kidney cadherin (K-cadherin)
N-sam; fibroblast growth factor receptor1 precursor (FGFR1); basic fibroblast growth factor receptor precursor (BFGFR); fms-like tyrosine kinase 2 (FLT2); heparin-binding growth factor receptor (HBGFR-alpha A1); HBGFR-alpha-A2; HBGFR-alpha-A3
glutathione S-transferase M4 (GSTM4)
insulin-like growth factor-binding protein 2 (IGF-binding protein 2; IGFBP2; IBP2)
metalloprotease/disintegrin/cysteine-rich protein precursor (MDC9)
mitogen-activated protein kinase 6 (MAP kinase 6; MAPK6; PRKM6); p97-MAPK; extracellular signal-regulated kinase 3 (ERK3)
6-O-methylguanine-DNA methyltransferase (MGMT); methylated-DNA-protein-cysteine methyltransferase
RAD50
caspase 9 precursor (CASP9); MCH6; ICE-like apoptotic protease 6 (ICE-LAP6); apoptotic protease activating factor 3 (APAF3)

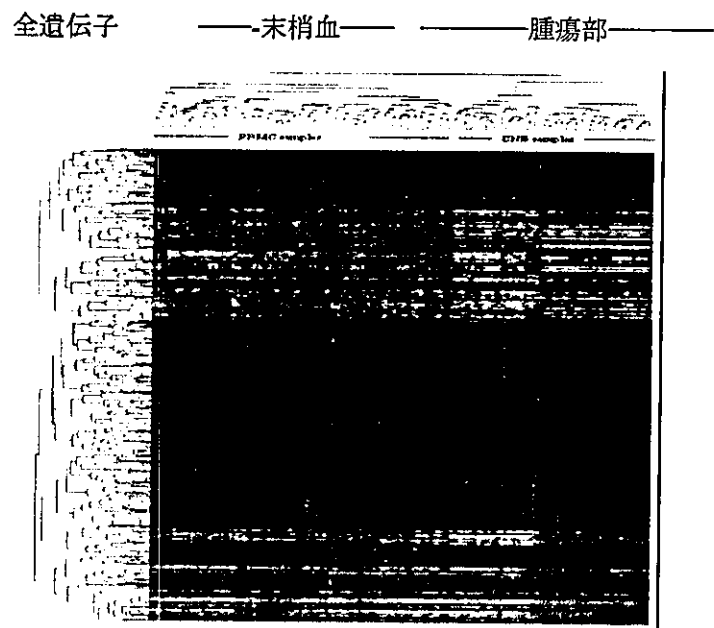
b) 乳癌で発現の低い遺伝子(PBMC との比較)

growth-arrest-specific protein 1 (GAS1)
selectin E precursor (SELE; ESEL); endothelial leukocyte adhesion molecule 1 (ELAM1)
phenylethanolamine N-methyltransferase (PNMTase); noradrenaline N-methyltransferase
ATP-binding cassette subfamily G (white) member 2 (ABCG2); breast cancer resistance protein (BCRP); mitoxantrone resistance protein 1 (MXR1)
caspase 3 (CASP3); apopain precursor; cysteine protease CPP32; YAMA protein; SREBP cleavage activity 1 protein (SCA1)
inhibitor of apoptosis protein1 (IAP1; API1) + IAP homolog C; TNFR2-TRAF signaling complex protein 1; MIHC
retinoblastoma-related protein p107
DNA polymerase alpha 70-kDa subunit (POLA2)
bleomycin hydrolase (BLM hydrolase)
cyclin-dependent kinase 4 inhibitor 2D (CDKN2D); p19-INK4D
glutathione S-transferase mu 1 (GSTM1; GST1); HB subunit 4; GTH4

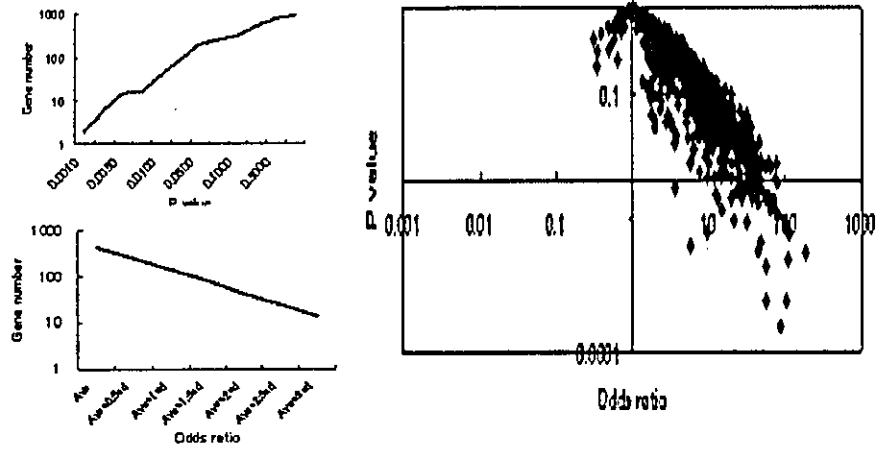
資料10 新カスタムアレイを用いた臨床サンプルの遺伝子発現解析（ホルモン関連遺伝子）。



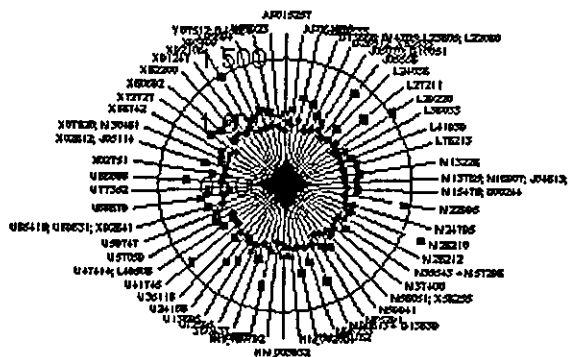
資料11 新カスタムアレイを用いた臨床サンプルの遺伝子発現解析（全遺伝子でのクラスタリング）



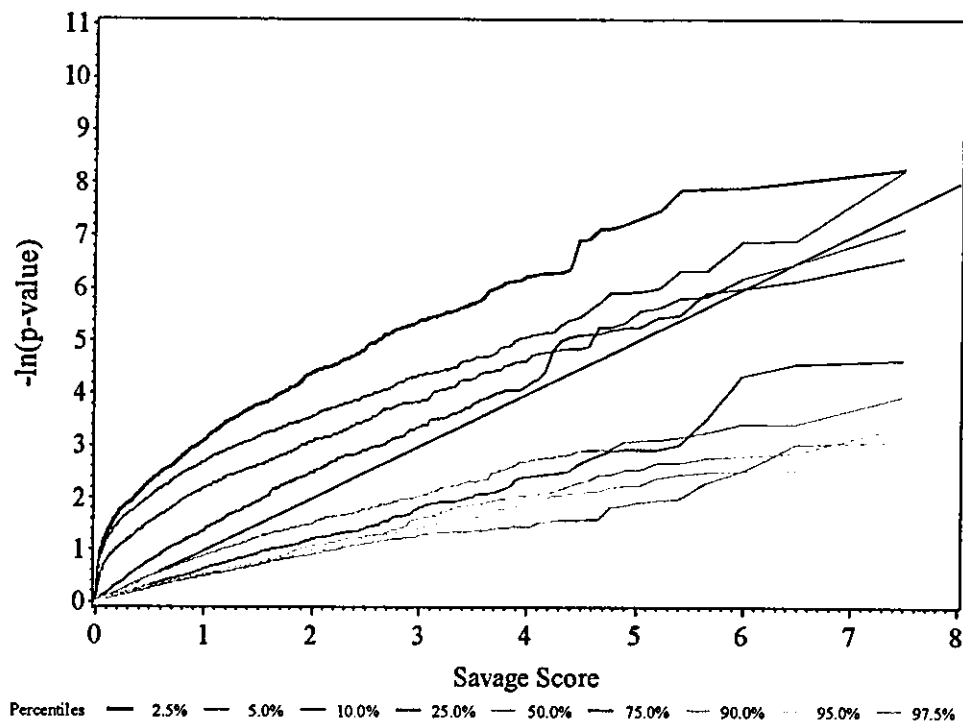
資料12 L3/L2サンプルにおけるP 値およびオッズ比と選択される遺伝子数（左）およびP 値-オッズ比の関係（右）

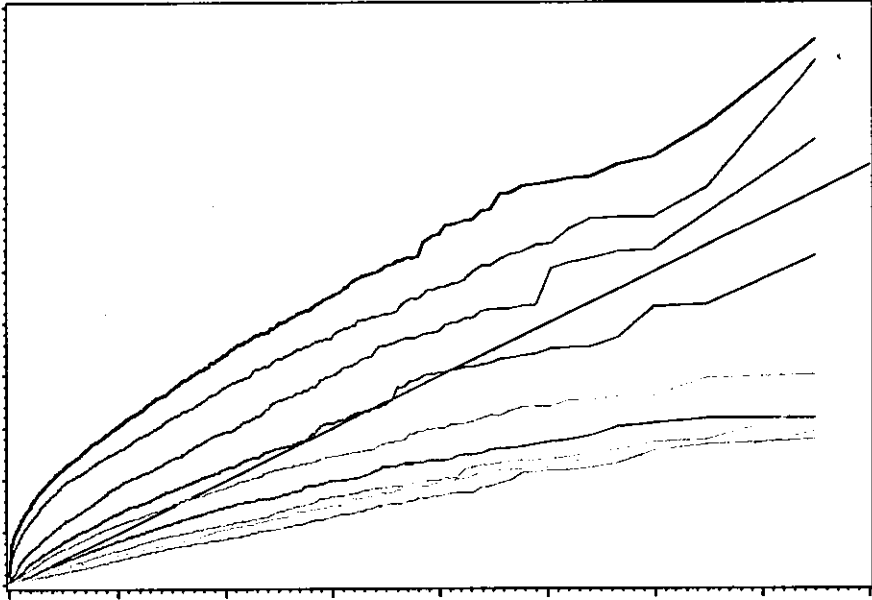


資料13 選択した各遺伝子の末梢神経障害グレード0または1の群と2の群とにおける発現状態。青菱形が0、1群を示し桃色正方形が2+群を示す。同心円内内側は発現変化量が低いことを示し外側が高いことを示す。



資料15 p値の負の対数の合計を指標とした場合の参照線





資料17. group 1および 2の遺伝子のAUCとオッズ比

group	GenBank Accession no.	AUC	Odds Ratios	2.5th Percentiles of Odds Ratio	97.5th Percentiles of Odds Ratio
1	Y00486	53.7	7.58	2.96	36.67
1	L19067	52.7	13.63	3.18	425.78
1	AF017786	49.0	6.99	1.83	88.34
1	AF011792	48.0	4.91	1.80	19.40
1	AF048700	46.8	3.29	1.28	15.85
1	X05030	46.2	0.71	0.32	0.92
2	M62402	43.7	6.60	2.50	38.65
2	X76220	43.5	2.44	1.44	4.79
2	U66879	42.7	0.22	0.01	0.83
2	M81934; S78187	42.7	4.36	1.85	14.37
2	U09178	40.0	3.38	1.55	10.60

* AUC: Area Under the Curve