

図2 Genechip

プローブ設計
 パーフェクトマッチ、ミスマッチの比較により
 クロスハイブリによる影響を軽減

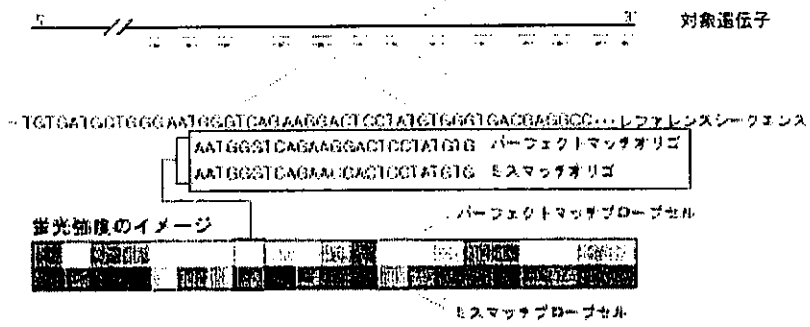


図3 Genechip

解析の流れ

サンプル

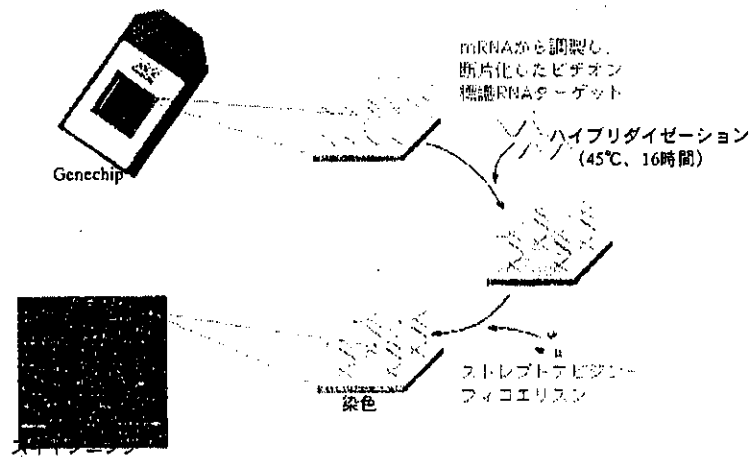


図 4

GenechipとcDNAマイクロアレイの違い

cDNAマイクロアレイでは・・・



スライドガラス上にDNAをスポットする
→スポッターがあれば、研究室単位で
実施可能（当部は所有していない）



競合ハイブリによる解析が主
2サンプルを競合させ同スライド上で
解析する

図 5

cDNAマイクロアレイにおけるシグナル検出感度向上

Tyramide Signal Amplification method

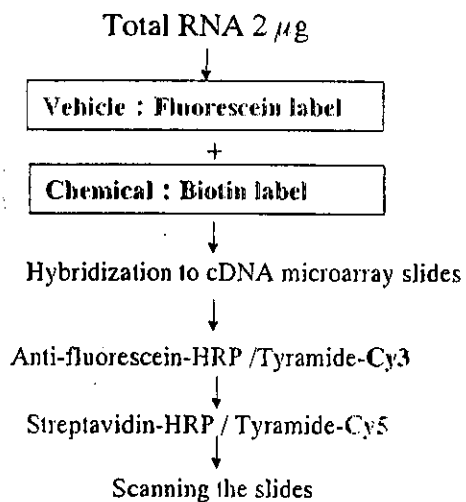
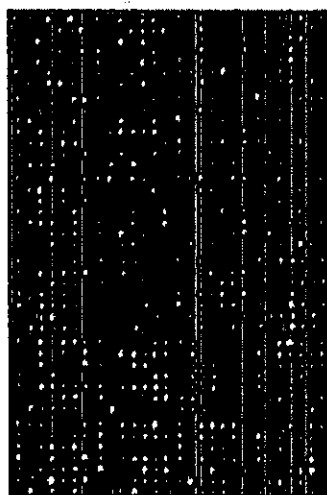
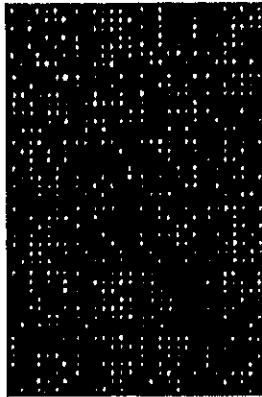


図 6

Auto hybridization

自動ハイブリ装置 (GeneTAC) とマニュアル法を比較検討した

Manual



Auto hybridize
(GeneTAC)

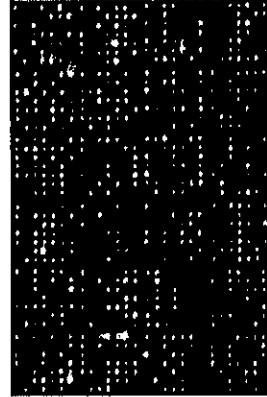


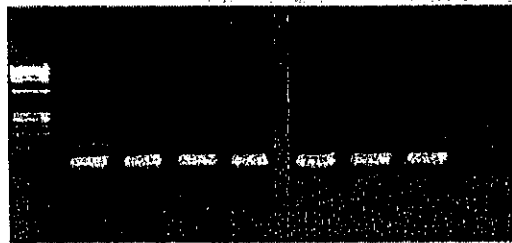
図 7

Specific amplification of spike RNA

Spike RNA (ng)	100	25	6.25	1.56	0.39	0.098	0.024	0
Liver RNA (μ g)	1	1	1	1	1	1	1	1

No amplification
with 0ng spike RNA

<Protocol>
Liver RNA 1 μ g
Spike RNA x ng
↓
cDNA synthesis
↓
PCR



Primers
FW: CACGCCTTAAACGGTGAAC
RV: GGAAAAGCCGCGACGAA

Cycle
(94°C, 30s → 60°C, 1m) x 35 → 4°C

図 8

Correlation between spike RNA ratio and tissue weight

組織重量とSpike RNA ratioが逆相関することが検証された

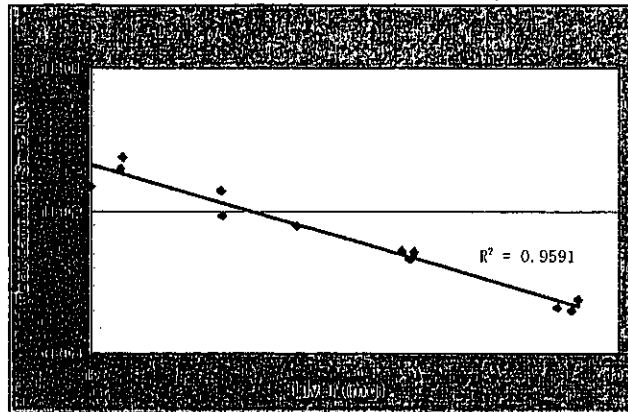


図 9

cDNAマイクロアレイ解析まとめ

(1) Genechipシステムによる解析

25merのオリゴDNAが高密度に配置されたGenechipを使用。

必要RNA量：total RNA 5 μ g/sample

対象種

ヒト	60,000以上の既知遺伝子とESTクラスター
ラット	24,000以上の既知遺伝子とESTクラスター
マウス	36,000以上の既知遺伝子とESTクラスター
酵母	既知ORF6,400すべてに対応

(2) スライドガラス型市販cDNAマイクロアレイによる解析

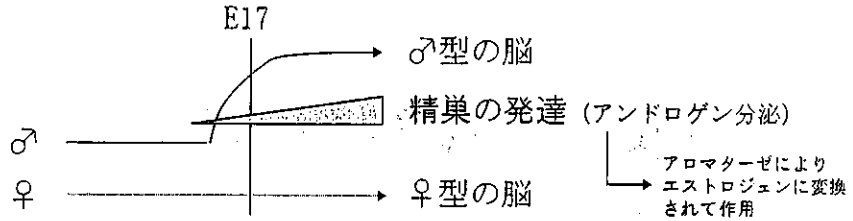
必要RNA量：total RNA 2 μ g/sample

対象種

ヒト、ラット、マウス、酵母等。
遺伝子種類は1000~4000遺伝子程度

図 1 0

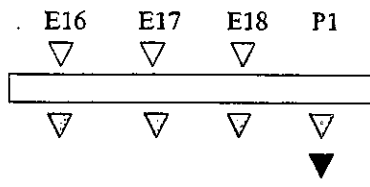
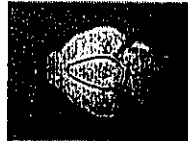
脳性分化



- ・発生に伴い視床下部においてエストロゲン作用下、雌雄で異なる遺伝子発現が起きている
 - ・発現の異なる遺伝子の機能解析により、視床下部脳性分化の分子メカニズム解析が可能となる
- ↓
- ・遺伝子発現パターンから視床下部発生に対するエストロゲン様作用を検出可能となる。
 - ・そのエストロゲン様作用により、視床下部発生に対しどのような影響が生じるかを予測可能となる。

図 1 1

実験計画



- ▽ : DES投与
0, 0.2, 2 $\mu\text{g}/\text{kg}/\text{day}$
- ▽ : サンプル分離
- ▼ : DESサンプル分離

<実験手順>

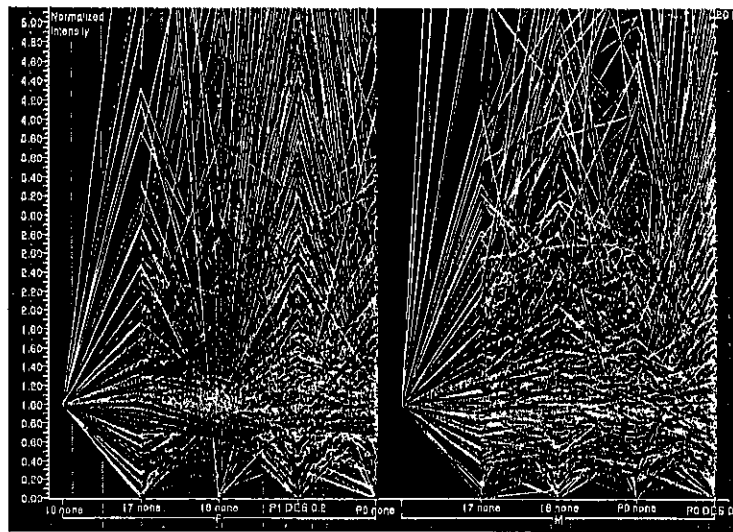
- 仔ごとに視床下部分離
- ↓
- 性別決定
- ↓
- プールしてRNA抽出
- ↓
- マイクロアレイ解析

<解析方針>

- 雌雄特異的発現を示す遺伝子群の抽出
- ↓
- DES投与によるパターンとの比較
- ↓
- DES視床下部発生影響の
遺伝子発現変化測定による評価可否判断

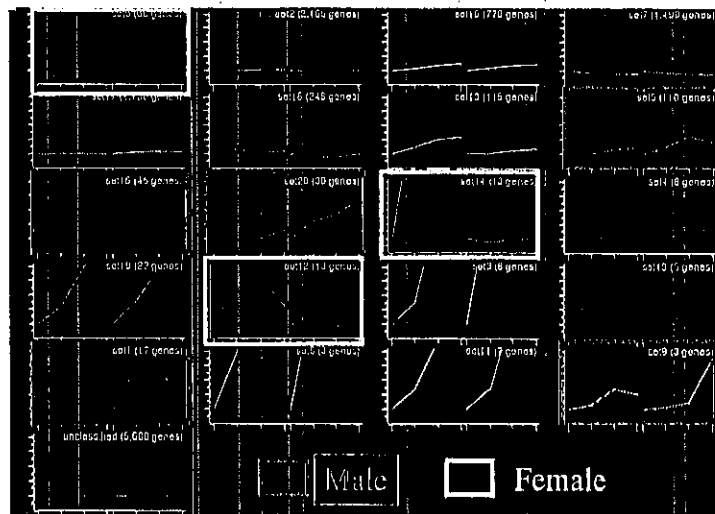
12

Time Course



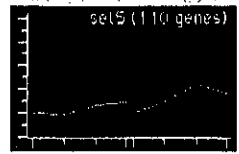
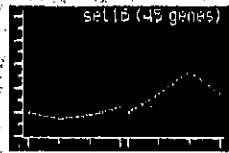
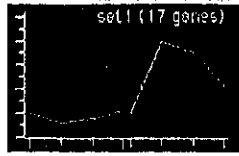
13

Cluster analysis



☒ 1 4

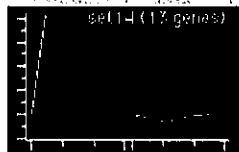
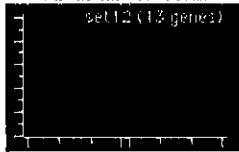
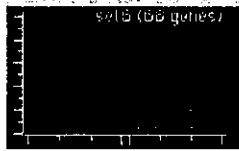
Male specific genes



set1	17
set5	110
set9	3
set16	45
set20	30
total	205

☒ 1 5

Female specific genes



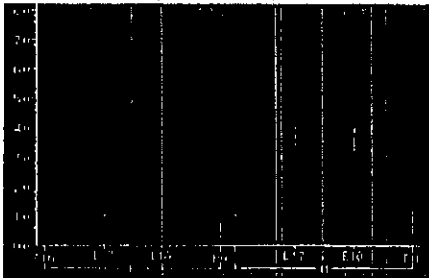
set6	68
set12	13
set14	13
total	94

☒ 1 6

Genes with sex specific expression patterns

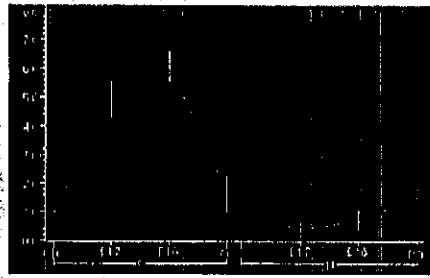
EST (AW061235)

477 bp
the mouse brain (cerebellum, brain
stems, olfactory bulbs, hypothalamus, cortex,
amygdala, basal ganglia, pineal gland,
striatum, hippocampus)



EST (AW120990)

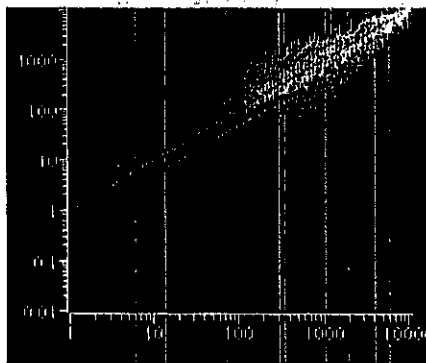
440 bp
the mouse brain (cerebellum, brain
stems, olfactory bulbs, hypothalamus, cortex,
amygdala, basal ganglia, pineal gland,
striatum, hippocampus)



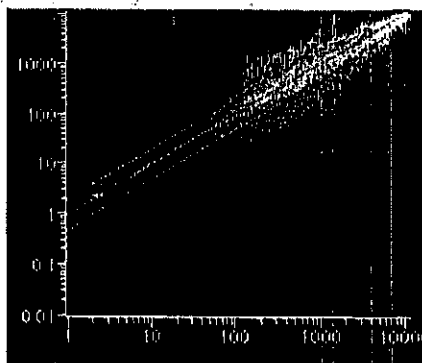
☒ 1 7

Change of gene expression induced by DES treatment

Male

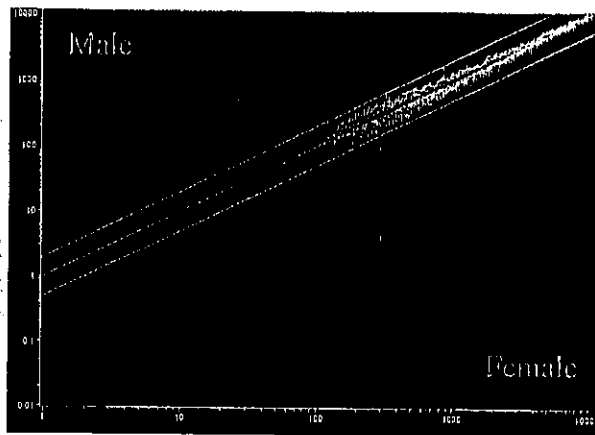


Female



☒ 18

Comparison of gene expression induced by DES treatment between male and female



☒ 19

Developmental expression of the genes induced in male by DES

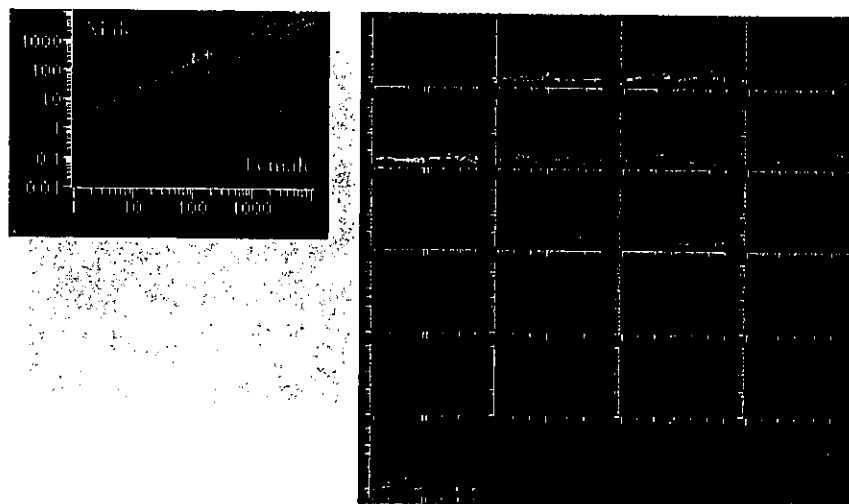
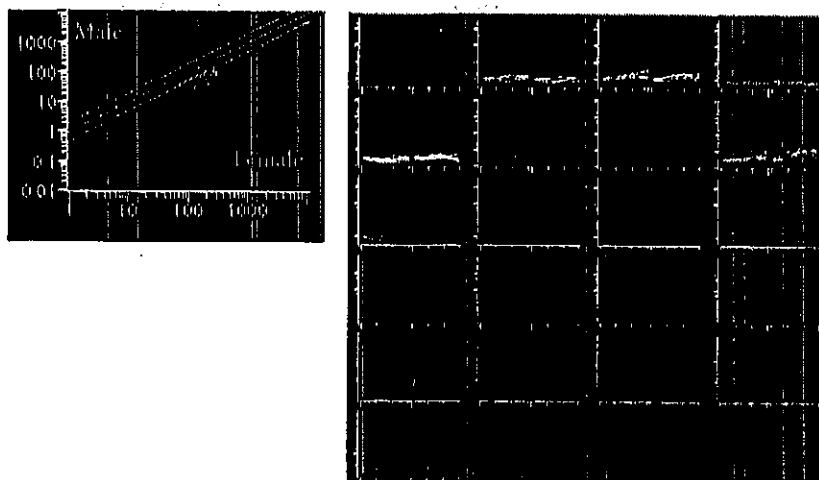


图 20

Developmental expression of the genes
induced in female by DES



別添6

研究成果の刊行に関する一覧表レイアウト

雑誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
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