Fig. 1A

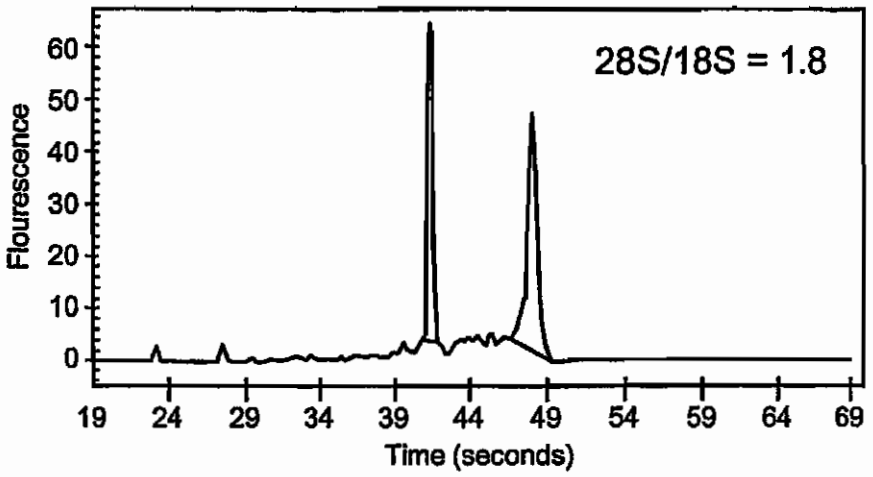


Fig. 1B

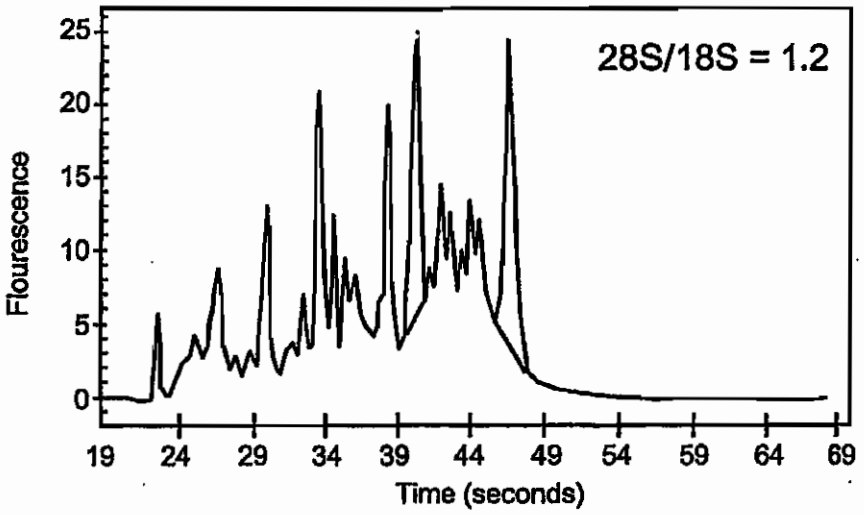
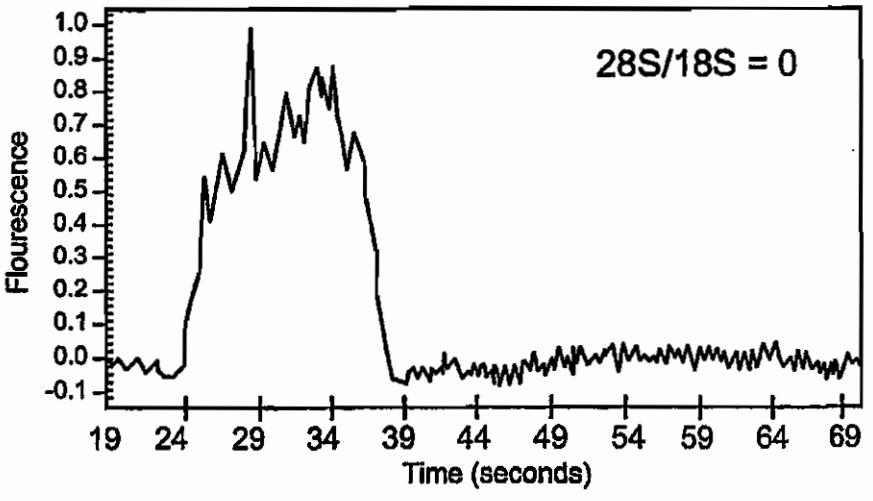


Fig. 1C



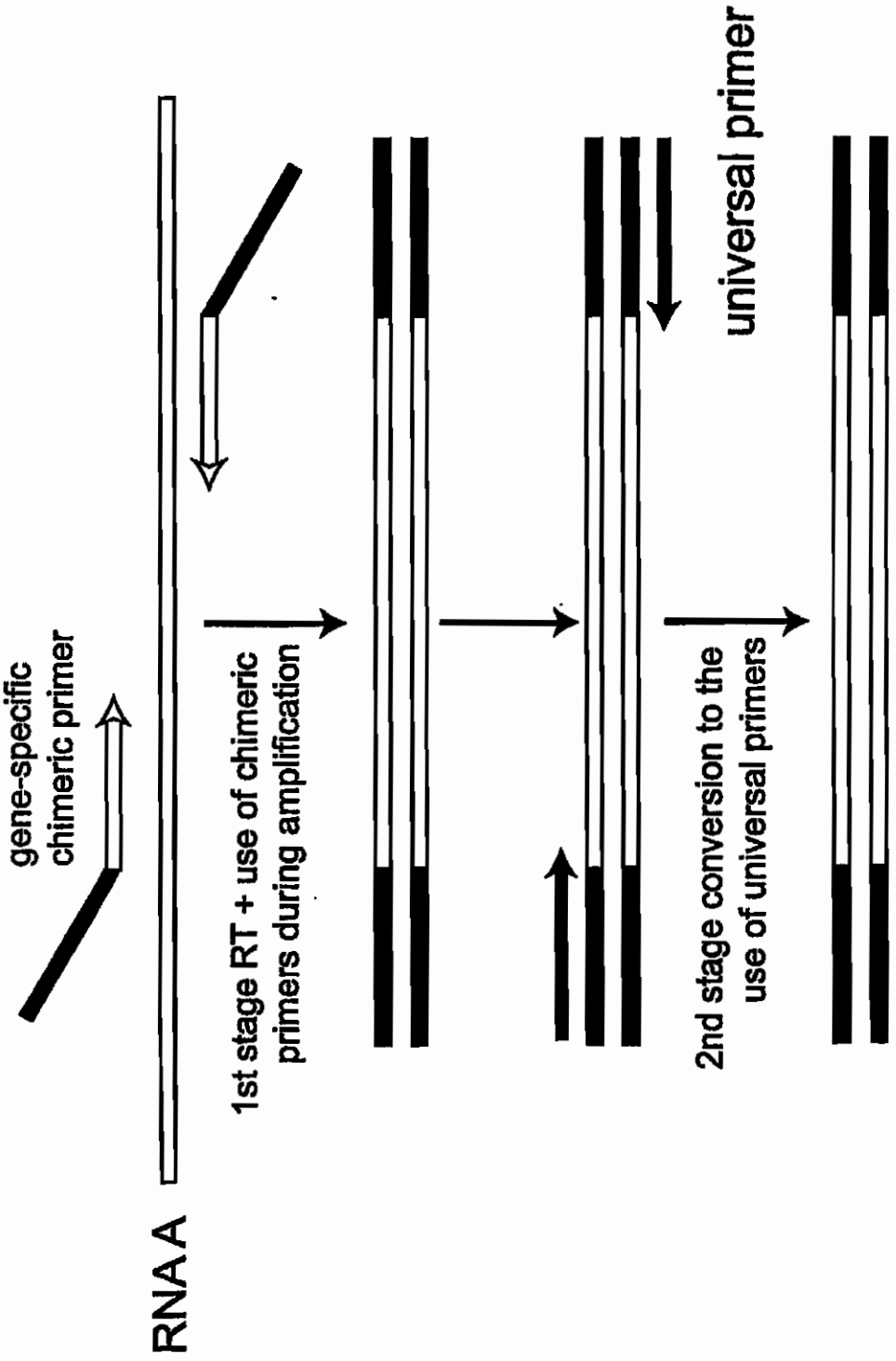

gene-specific
chimeric primer
RNA
1st stage RT + use of chimeric
primers during amplification
universal primer
2nd stage conversion to the
use of universal primers

Fig. 2

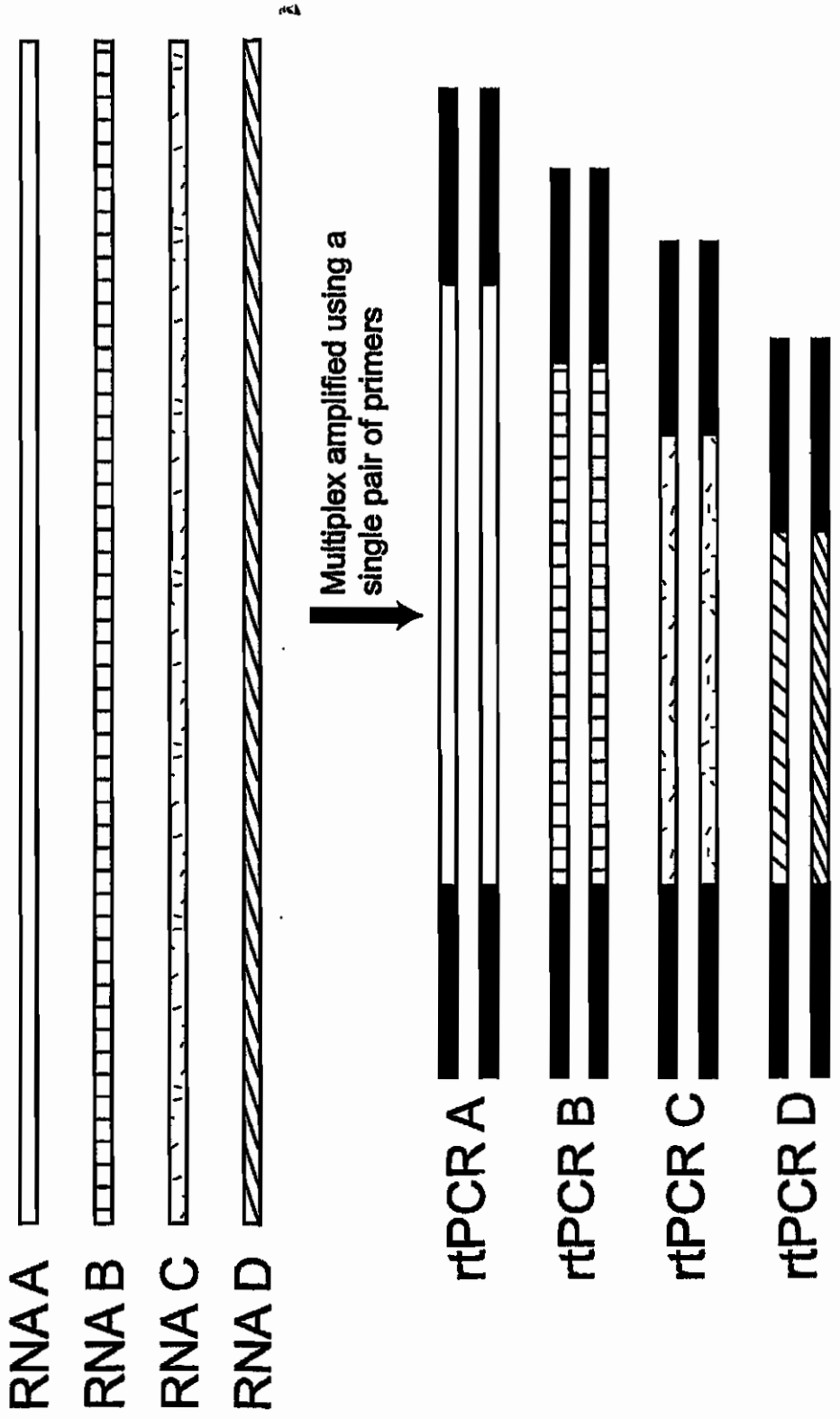

RNA A
RNA B
RNA C
RNA D
Multiplex amplified using a single pair of primers
rtPCR A
rtPCR B
rtPCR C
rtPCR D

Fig. 3

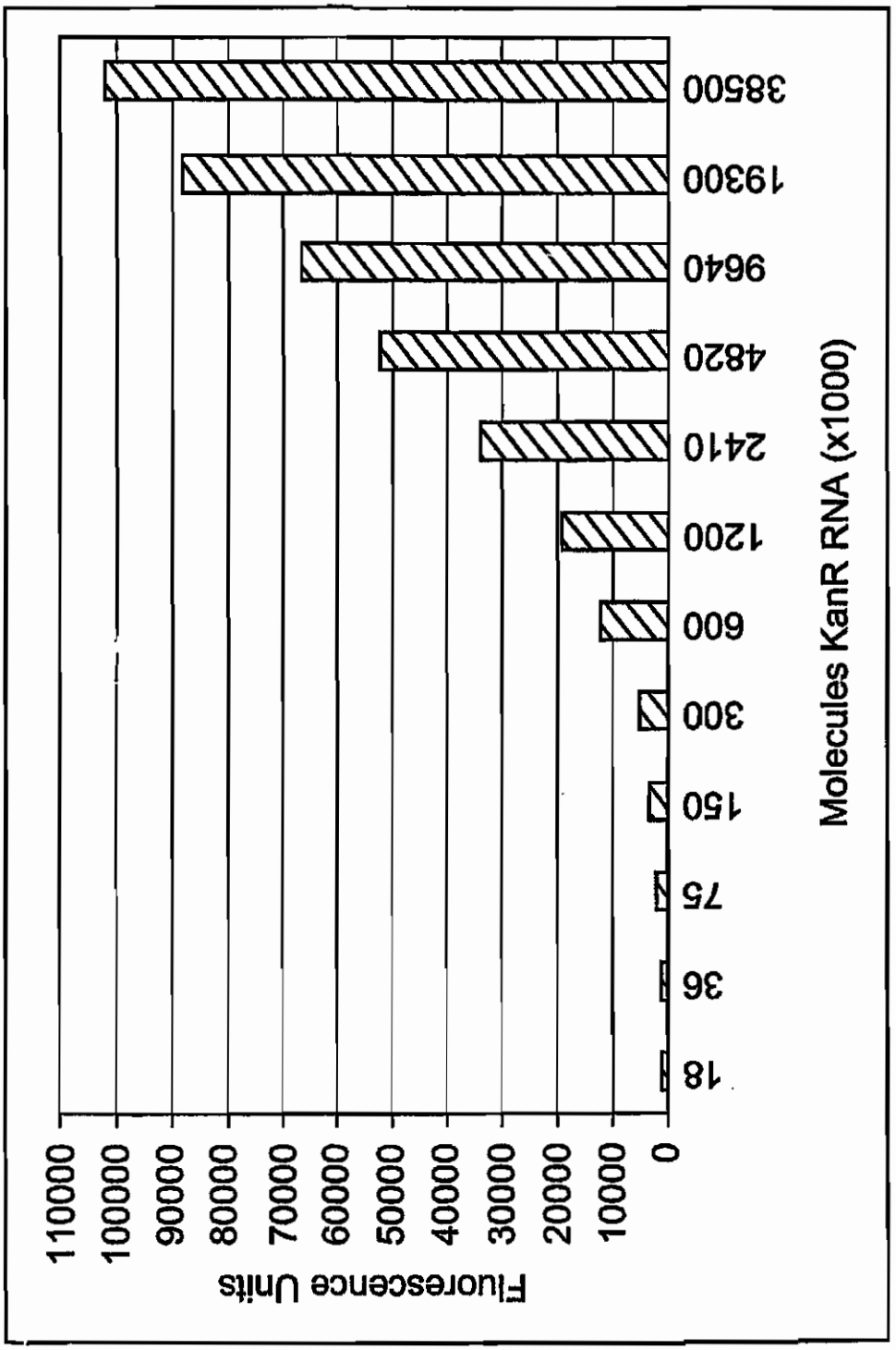

Fluorescence Units
0
10000
20000
30000
40000
50000
60000
70000
80000
90000
100000
110000
Molecules KanR RNA (x1000)
18
36
75
150
300
600
1200
2410
4820
9640
19300
38500

Fig. 4

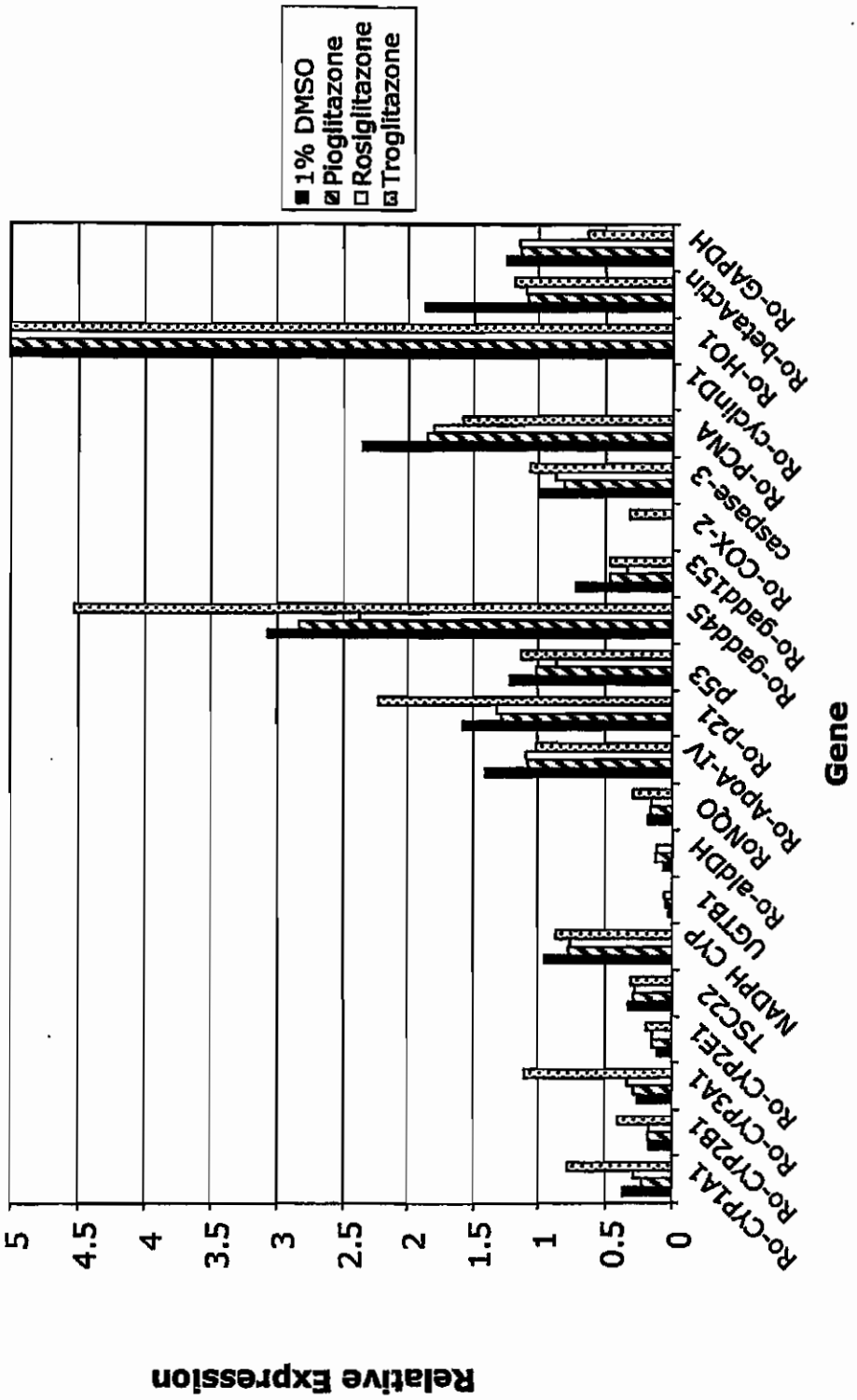

1% DMSO
Pioglitazone
Rosiglitazone
Troglitazone
Relative Expression
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Ro-CYP1A1
Ro-CYP2B1
Ro-CYP3A1
Ro-CYP2E1
TSC22
NADPH CYP
UGTB1
Ro-aldDH
RoNQO
Ro-ApoA-IV
Ro-p21
p53
Ro-gadd45
Ro-gadd153
Ro-COX-2
caspase-3
Ro-PCNA
Ro-cyclinD1
Ro-HO1
Ro-betaActin
Ro-GAPDH
Gene

Fig. 5

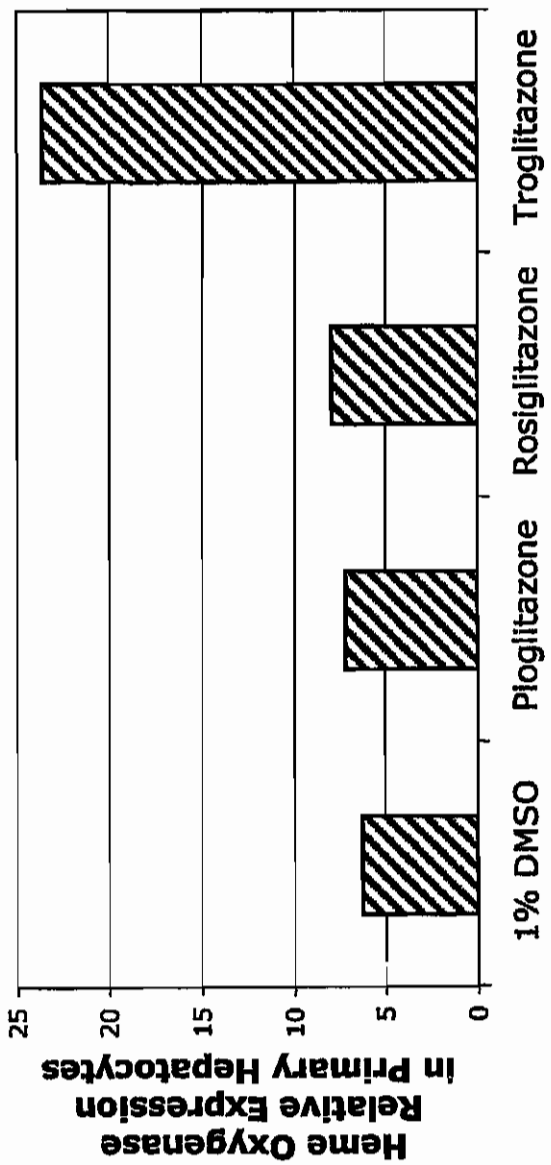

Heme Oxygenase
Relative Expression
in Primary Hepatocytes
25
20
15
10
5
0
1% DMSO
Pioglitazone
Rosiglitazone
Troglitazone
Treatment

Fig. 6

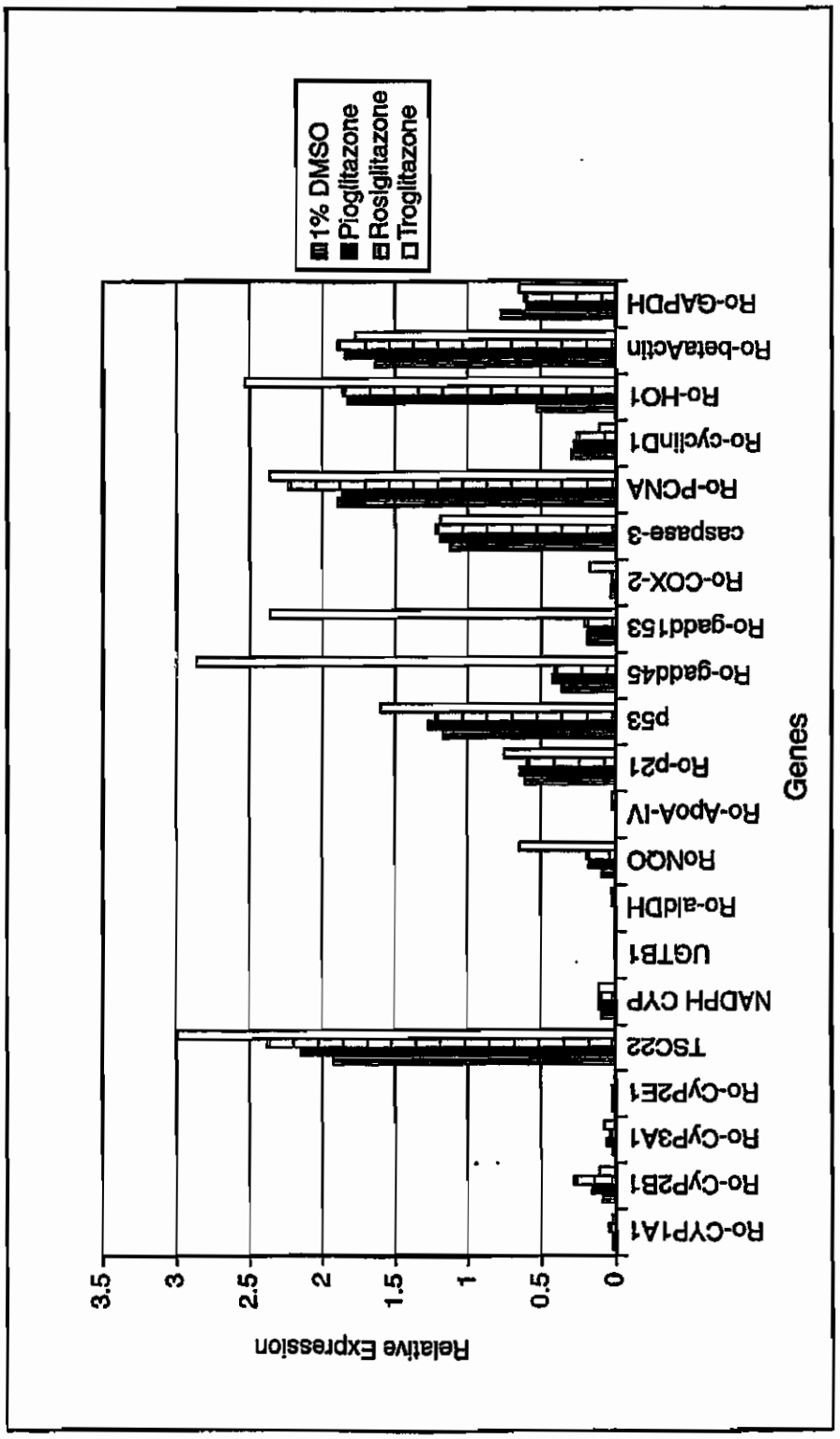

1% DMSO
Pioglitazone
Rosiglitazone
Troglitazone
Relative Expression
3.5
3
2.5
2
1.5
1
0.5
0
Genes
Ro-CYP1A1
Ro-CyP2B1
Ro-CyP3A1
Ro-CyP2E1
TSC22
NADPH CYP
UGTB1
Ro-aldDH
RoNQO
Ro-ApoA-IV
Ro-p21
p53
Ro-gadd45
Ro-gadd153
Ro-COX-2
caspase-3
Ro-PCNA
Ro-cyclinD1
Ro-HO1
Ro-betaActin
Ro-GAPDH

Fig. 7

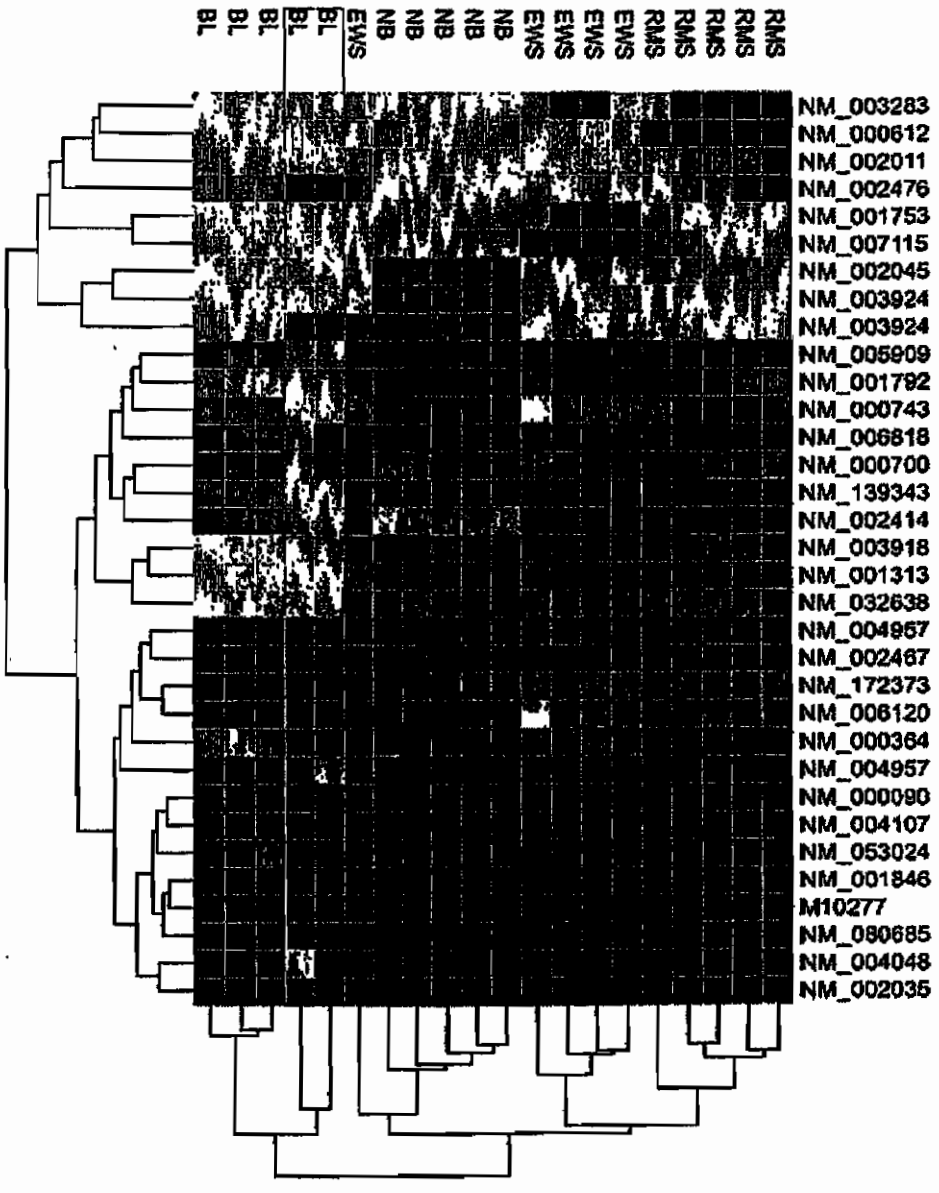

BL
BL
BL
BL
BL
EWS
NB
NB
NB
NB
NB
EWS
EWS
EWS
EWS
RMS
RMS
RMS
RMS
RMS
NM_003283
NM_000612
NM_002011
NM_002476
NM_001753
NM_007115
NM_002045
NM_003924
NM_003924
NM_005909
NM_001792
NM_000743
NM_006818
NM_000700
NM_139343
NM_002414
NM_003918
NM_001313
NM_032638
NM_004967
NM_002467
NM_172373
NM_006120
NM_000364
NM_004957
NM_000090
NM_004107
NM_053024
NM_001846
M10277
NM_080685
NM_004048
NM_002035

Fig. 8

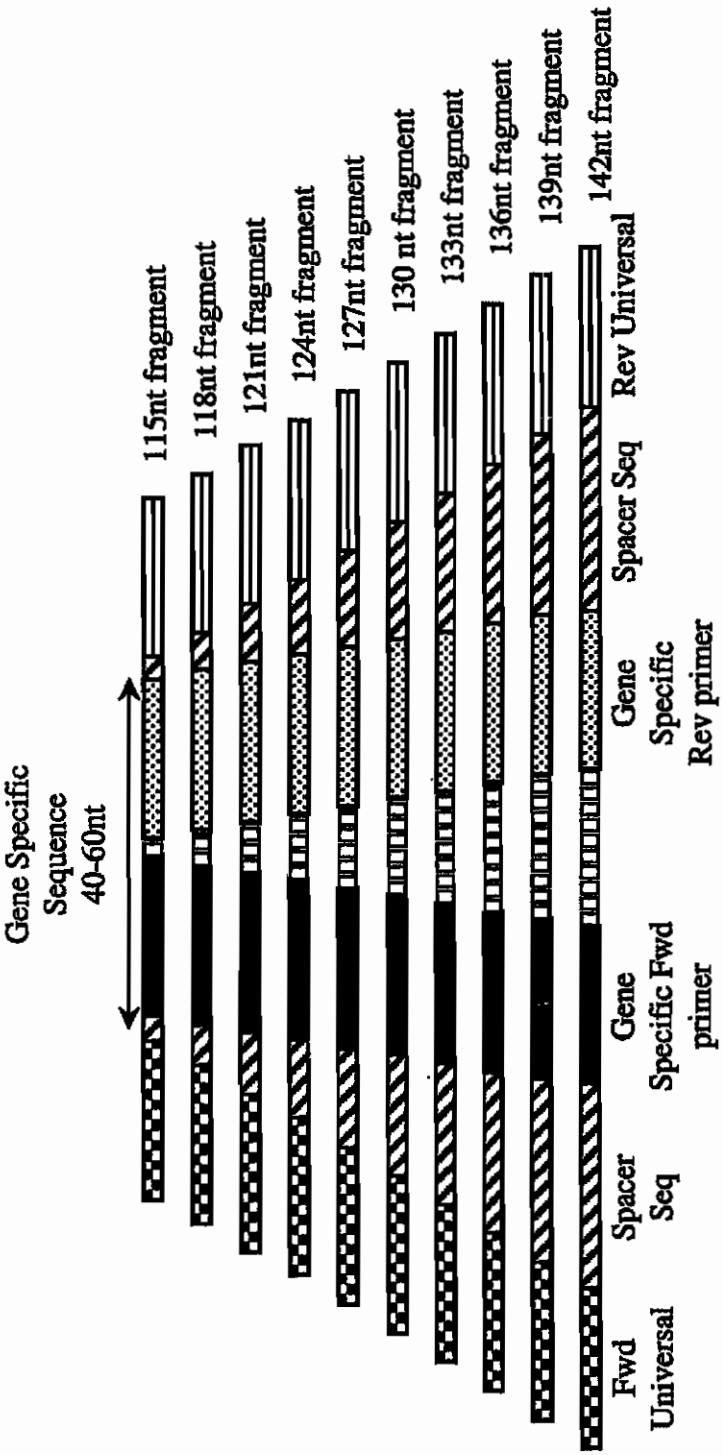

Gene Specific Sequence 40-60nt
115nt fragment
118nt fragment
121nt fragment
124nt fragment
127nt fragment
130 nt fragment
133nt fragment
136nt fragment
139nt fragment
142nt fragment
Fwd Universal
Spacer Seq
Gene Specific Fwd primer
Gene Specific Rev primer
Spacer Seq
Rev Universal

Fig. 9

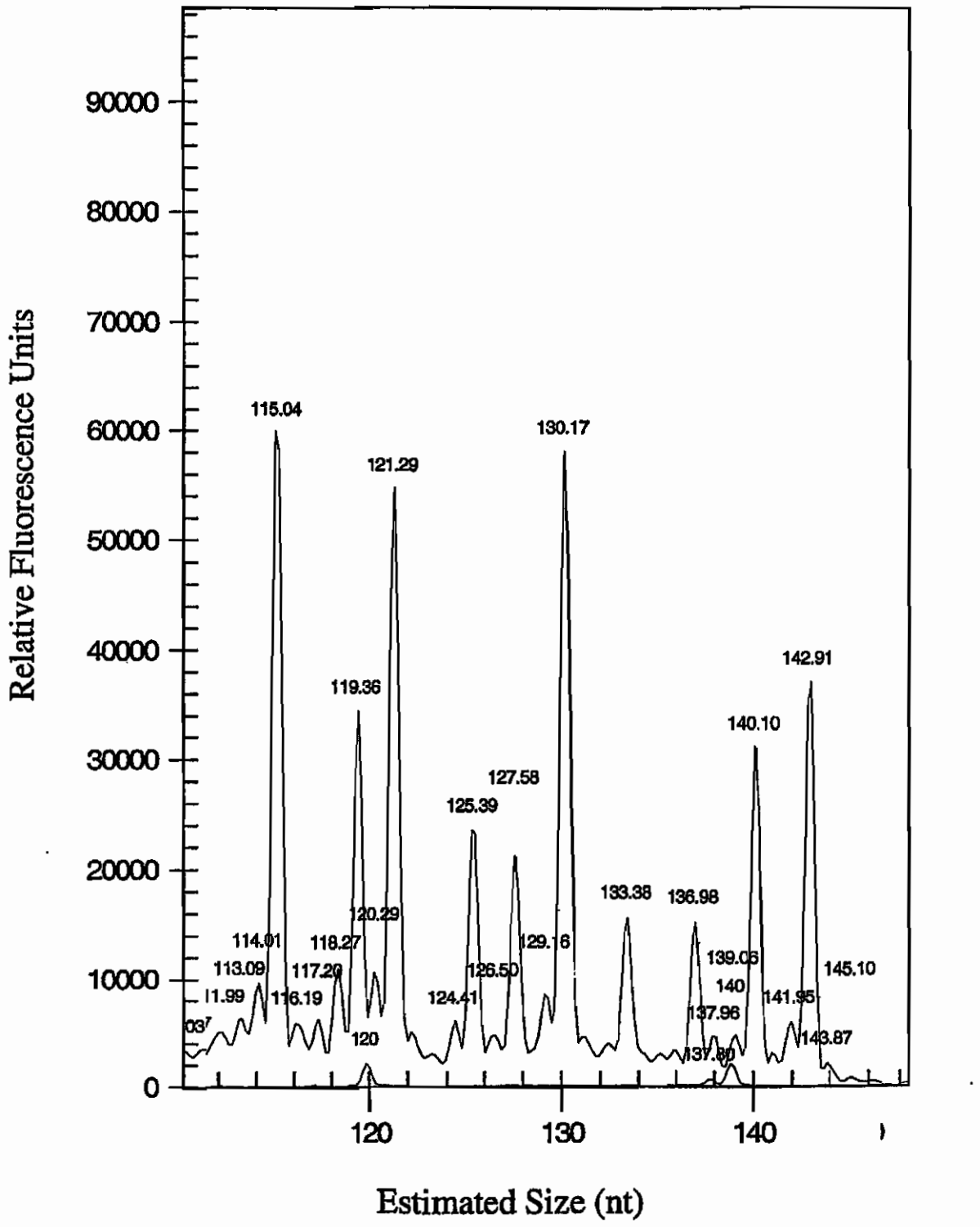

Relative Fluorescence Units
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
115.04
121.29
130.17
119.36
142.91
140.10
127.58
125.39
133.38
136.98
120.29
114.01
118.27
129.16
139.05
145.10
113.09
117.20
126.50
140
141.95
124.41
137.96
143.87
120
130
140
Estimated Size (nt)

Fig. 10

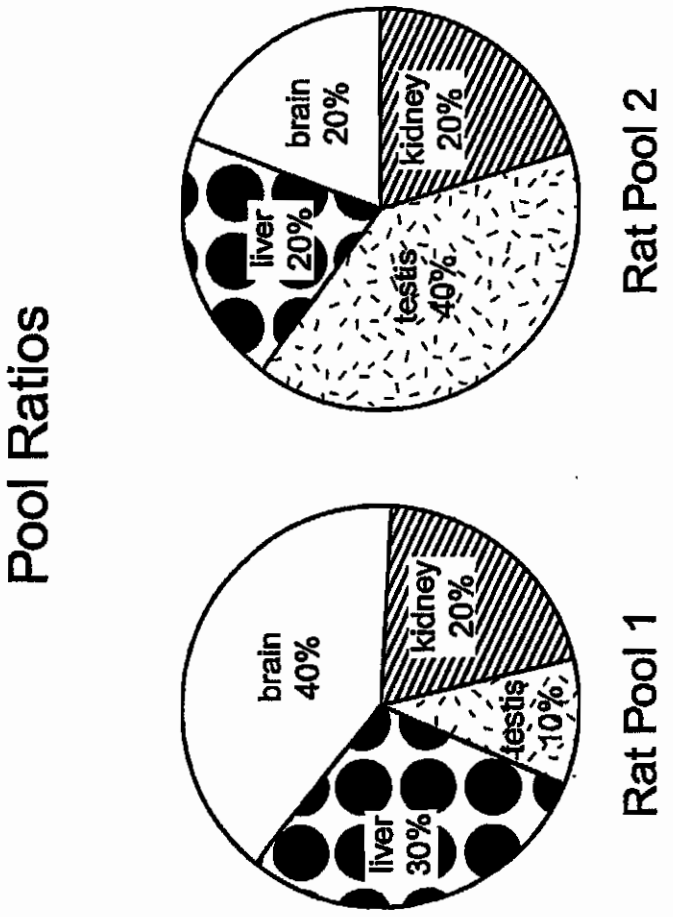

Pool Ratios
brain 20%
kidney 20%
liver 20%
testis 40%
Rat Pool 2
brain 40%
kidney 20%
testis 10%
liver 30%
Rat Pool 1

Fig. 11

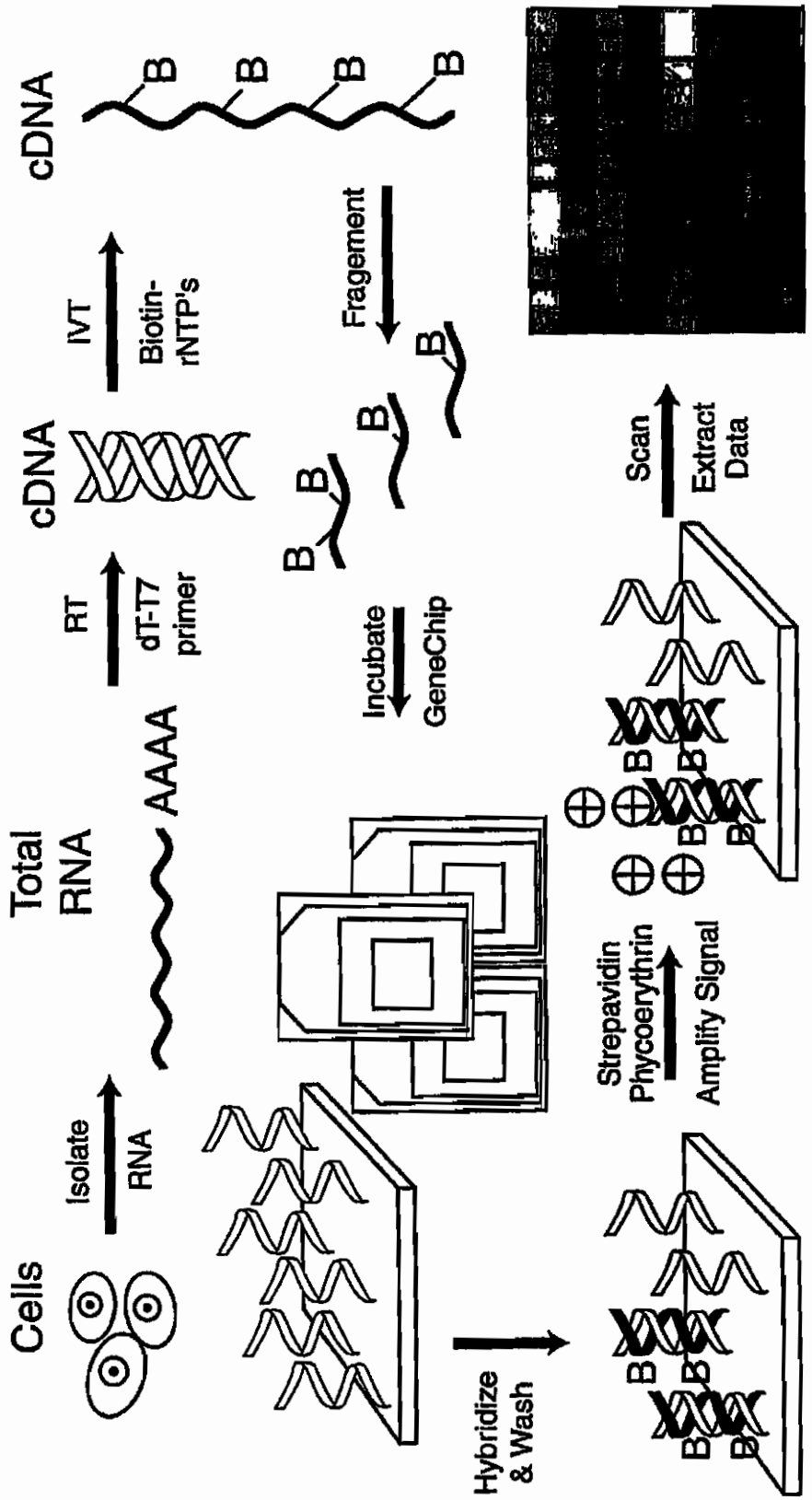

Cells
Isolate RNA
Total RNA
AAAA
RT
dT-T7 primer
cDNA
IVT
Biotin-rNTP's
cDNA
B
Fragement
Incubate GeneChip
Hybridize & Wash
Strepavidin Phycoerythrin
Amplify Signal
Scan
Extract Data

Fig. 12

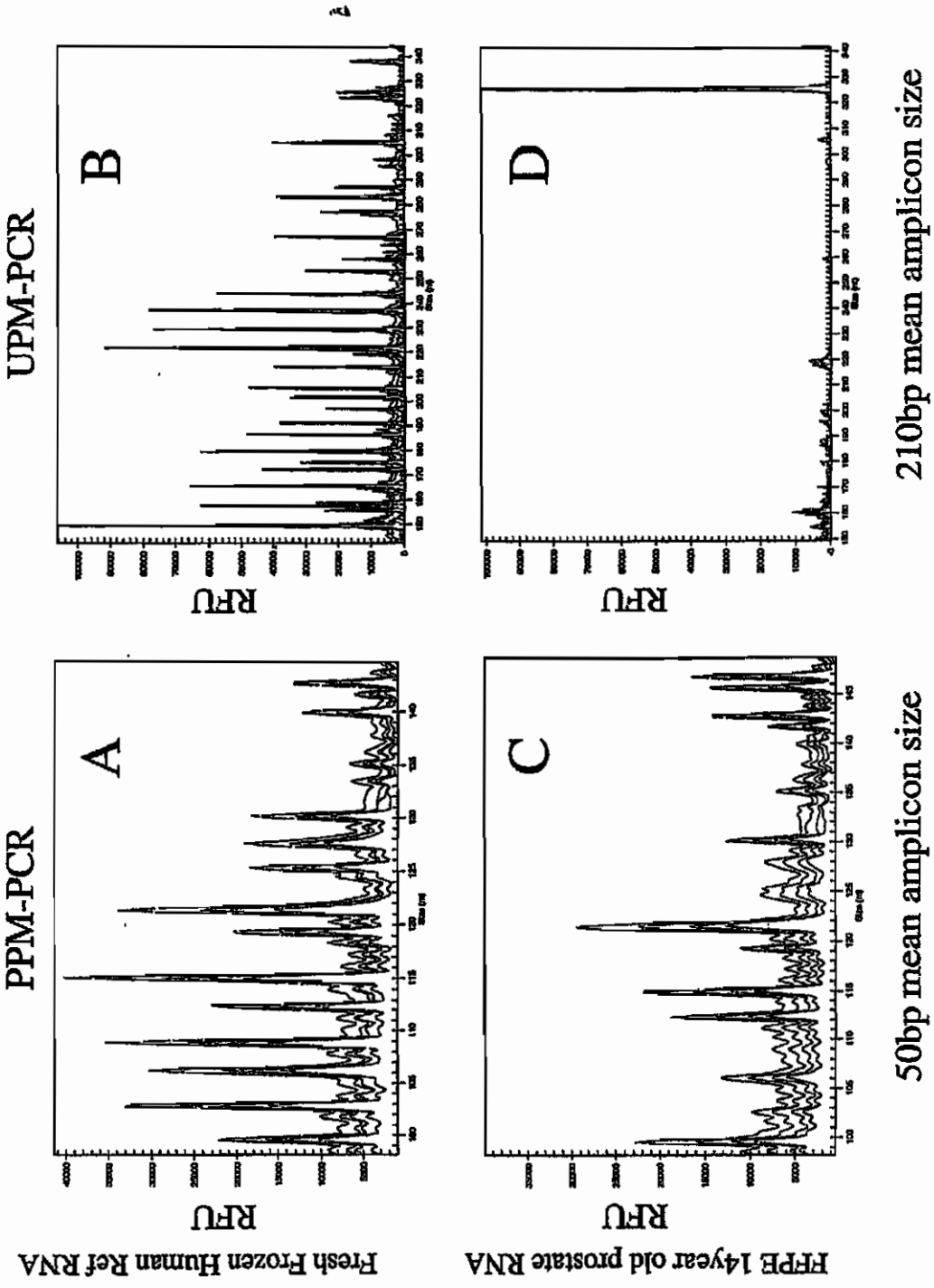

PPM-PCR
UPM-PCR
A
B
C
D
RFU
Fresh Frozen Human Ref RNA
FFPE 14year old prostate RNA
50bp mean amplicon size
210bp mean amplicon size

Fig. 13

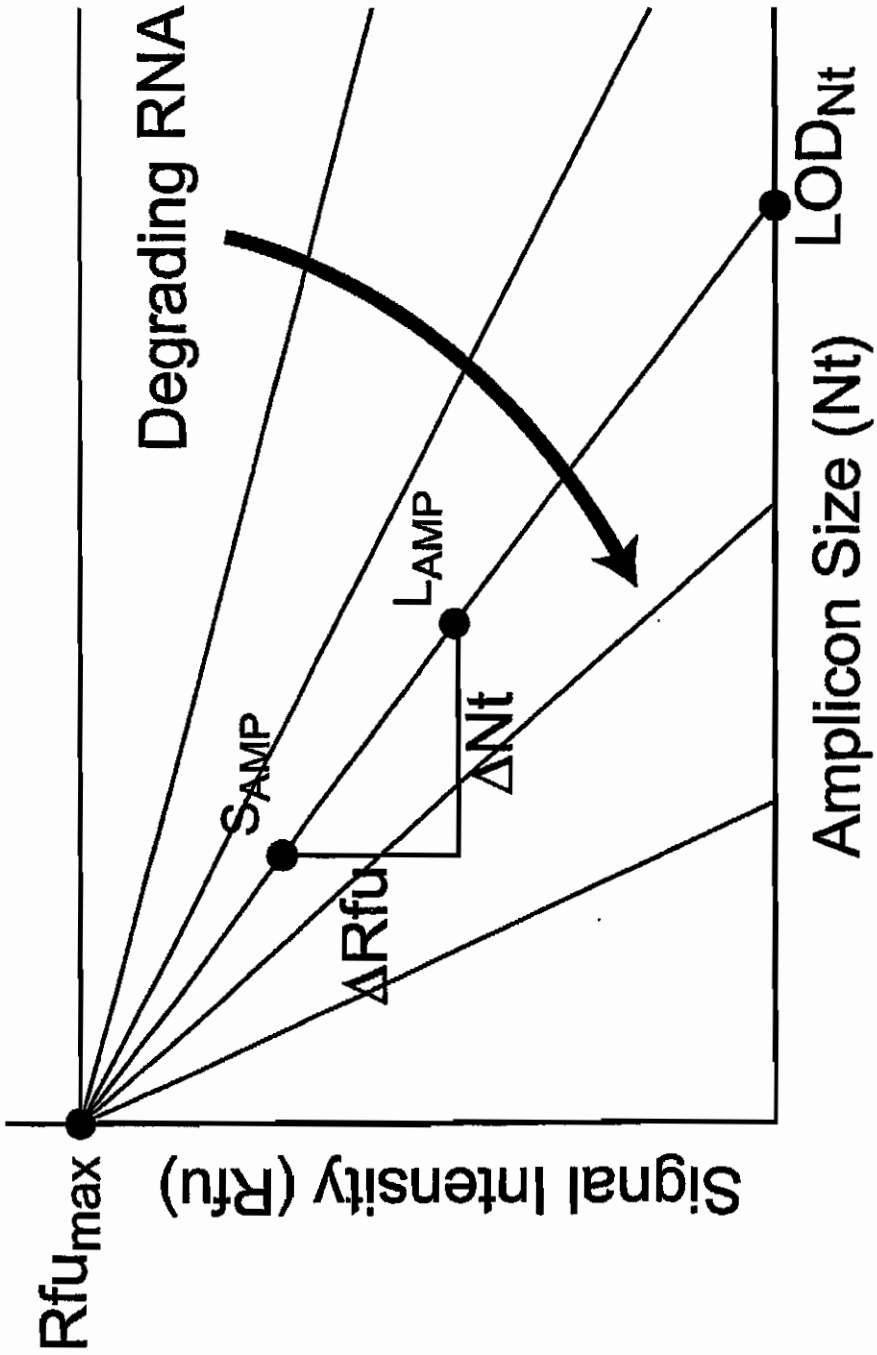

Degrading RNA
LOD$_{Nt}$
L$_{AMP}$
S$_{AMP}$
ΔNt
ΔRfu
Amplicon Size (Nt)
Signal Intensity (Rfu)
Rfu$_{max}$

Fig. 14

Gene	Low Dose Drug Treatment		Mid Dose Drug Treatment		High Dose Drug Treatment			
	Fold Change in Gene Expression Compared to Pooled Control RNAs							
ApoC-III	-2.3	-1.1	-2	-2.1	-2.7	-2.5	UD	UPM-PCR
	-1.6	-1.8	-1.5	-2.2	-2.7	-2.4	-4.7	-4.6	Microarray
HS-SULT	-2	-3.2	-2.3	-5.9	-2	-7.8	-2.3	-3.4	UD	UPM-PCR
	-1.6	-2.1	-2.1	-3.9	-1.4	-2.8	-2.1	-2.5	-3.9	Microarray
PDK4	6.7	7.3	7.8	8.8	9.4	8.1	10.2	11.2	UD	UPM-PCR UP-rtPCR
	9.4	9.4	8.9	14.4	11.8	13.9	12.9	16.6	18.5	Microarray
PEPCK	-4.1	-7.6	-4.1	-3.3	UD	UD	-1.4	-5.5	UD	UPM-PCR UP-rtPCR
	-2.7	-3.4	-2.3	-2	-3.5	-3.7	-1.6	-2.4	-2.7	Microarray
FACO	2.7	3.4	3.1	3.6	3.5	4.2	4.1	5.6	UD	UPM-PCR UP-rtPCR
	2.3	2.9	2.4	3.7	2.7	4	3.7	6	4.9	Microarray
TSC-22	-1.8	-2	-1.8	-2.5	-2.7	-2.2	-2.4	-2.9	UD	UPM-PCR UP-rtPCR
	-2.8	-2.9	-2.6	-3.7	-3.7	-2.6	-4	-5	-4.3	Microarray
IIbSPT	2.6	3.6	3.5	2.1	1.4	5.4	2.6	3.2	UD	UPM-PCR
	2.7	3.4	3.6	2.3	1.8	7	1.9	2	2.3	Microarray
catalase	1.1	1.1	1.2	1.1	1.2	1	1.2	1.3	UD	UPM-PCR
	UD	UD	UD	UD	UD	UD	UD	UD	UD	Microarray

Fig. 15

Tissue-Specific Genes

Gene	Tissue	Pool 1 / Pool 2	Expected Log2Ratio	Observed Log2Ratio Study A	Observed Log2Ratio Study B
Nx17	Kidney	No Change	0.00	-0.03	-0.05
Lipc	Liver	1.5 increase	0.58	0.56	0.44
Nfl	Brain	2.0 increase	1.00	1.19	1.02
Atp1a4	Testis	4.0 decrease	-2.00	-2.08	-2.06

Fig. 16

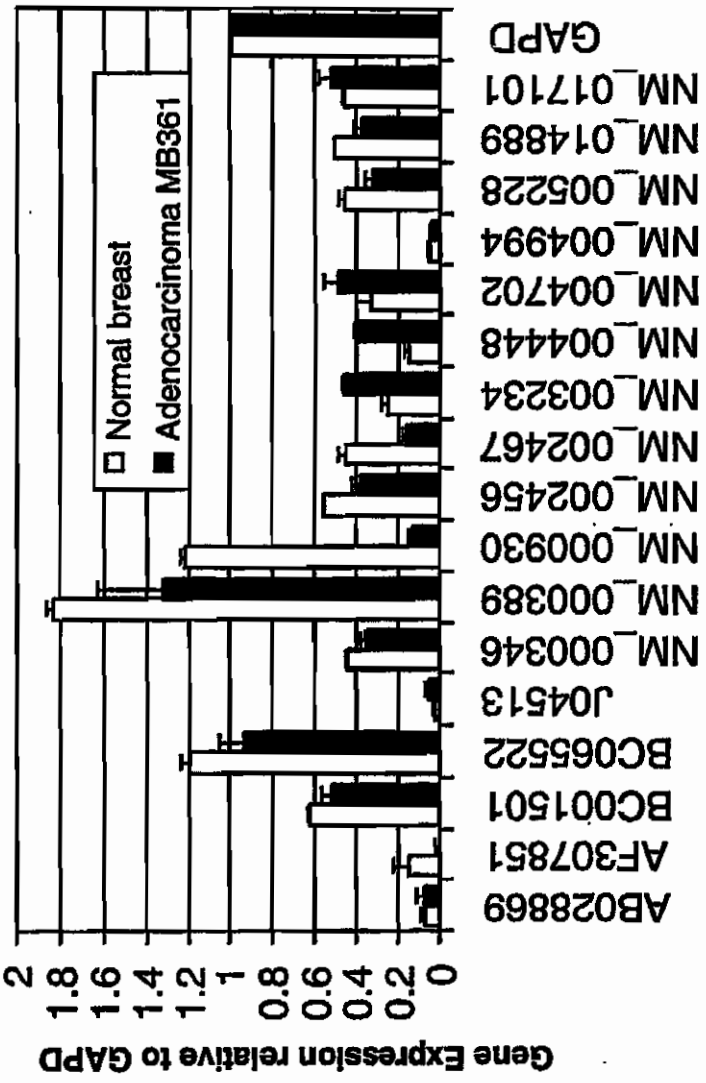

Normal breast
Adenocarcinoma MB361
Gene Expression relative to GAPD
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AB028869
AF307851
BC001501
BC065522
J04513
NM_000346
NM_000389
NM_000930
NM_002456
NM_002467
NM_003234
NM_004448
NM_004702
NM_004994
NM_005228
NM_014889
NM_017101
GAPD

Fig. 17