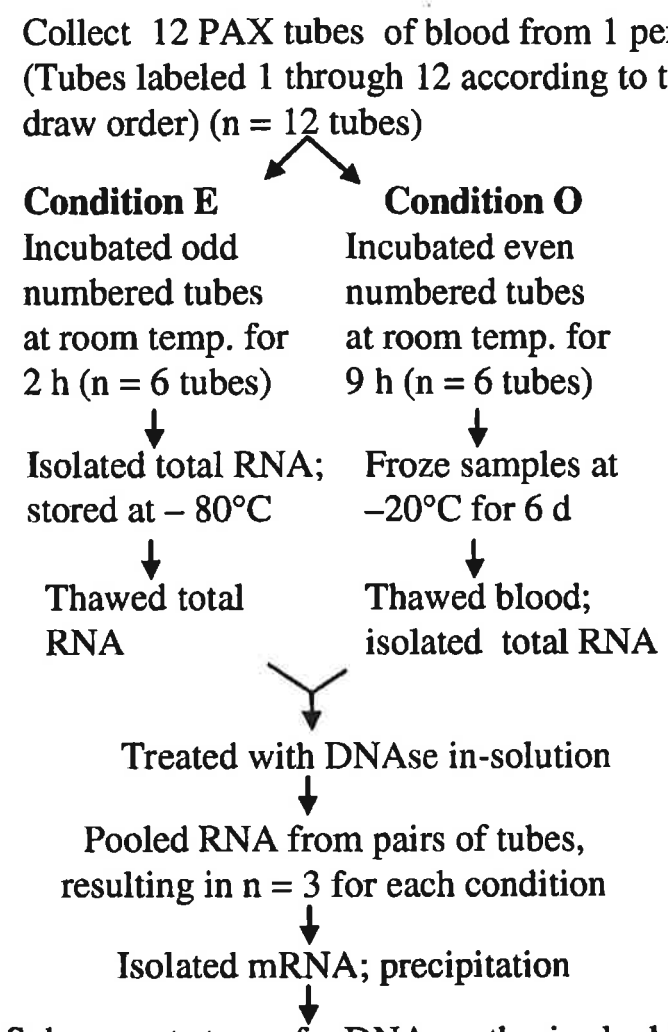

Collect 12 PAX tubes of blood from 1 person (Tubes labeled 1 through 12 according to the draw order) (n = 12 tubes)
Condition E
Incubated odd numbered tubes at room temp. for 2 h (n = 6 tubes)
Isolated total RNA; stored at – 80°C
Thawed total RNA
Condition O
Incubated even numbered tubes at room temp. for 9 h (n = 6 tubes)
Froze samples at –20°C for 6 d
Thawed blood; isolated total RNA
Treated with DNAse in-solution
Pooled RNA from pairs of tubes, resulting in n = 3 for each condition
Isolated mRNA; precipitation
Subsequent steps of: cDNA synthesis, double stranded cDNA synthesis, *in vitro* transcription, cRNA fragmentation, hybridization, and detection, were performed as described in the GeneChip Expression Analysis manual

Figure 1

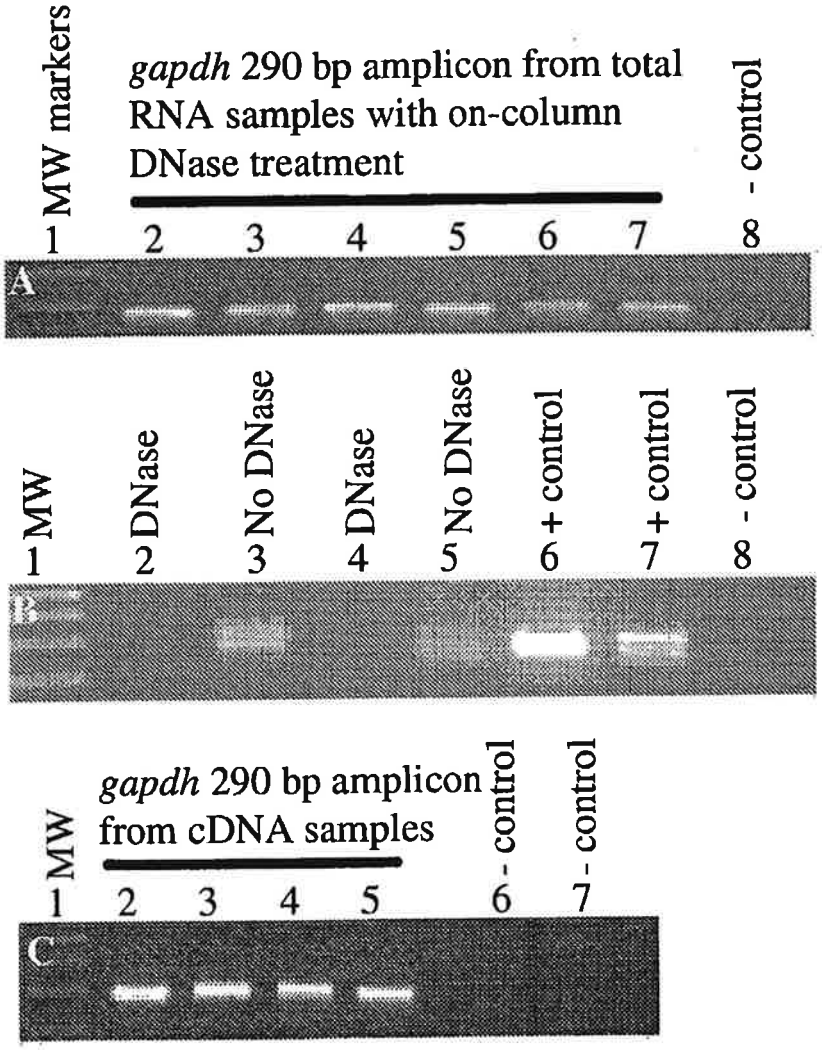

MW markers
gapdh 290 bp amplicon from total RNA samples with on-column DNase treatment
- control
1 2 3 4 5 6 7 8
A
MW
DNase
No DNase
DNase
No DNase
+ control
+ control
- control
1 2 3 4 5 6 7 8
B
MW
gapdh 290 bp amplicon from cDNA samples
- control
- control
1 2 3 4 5 6 7
C

Figure 2

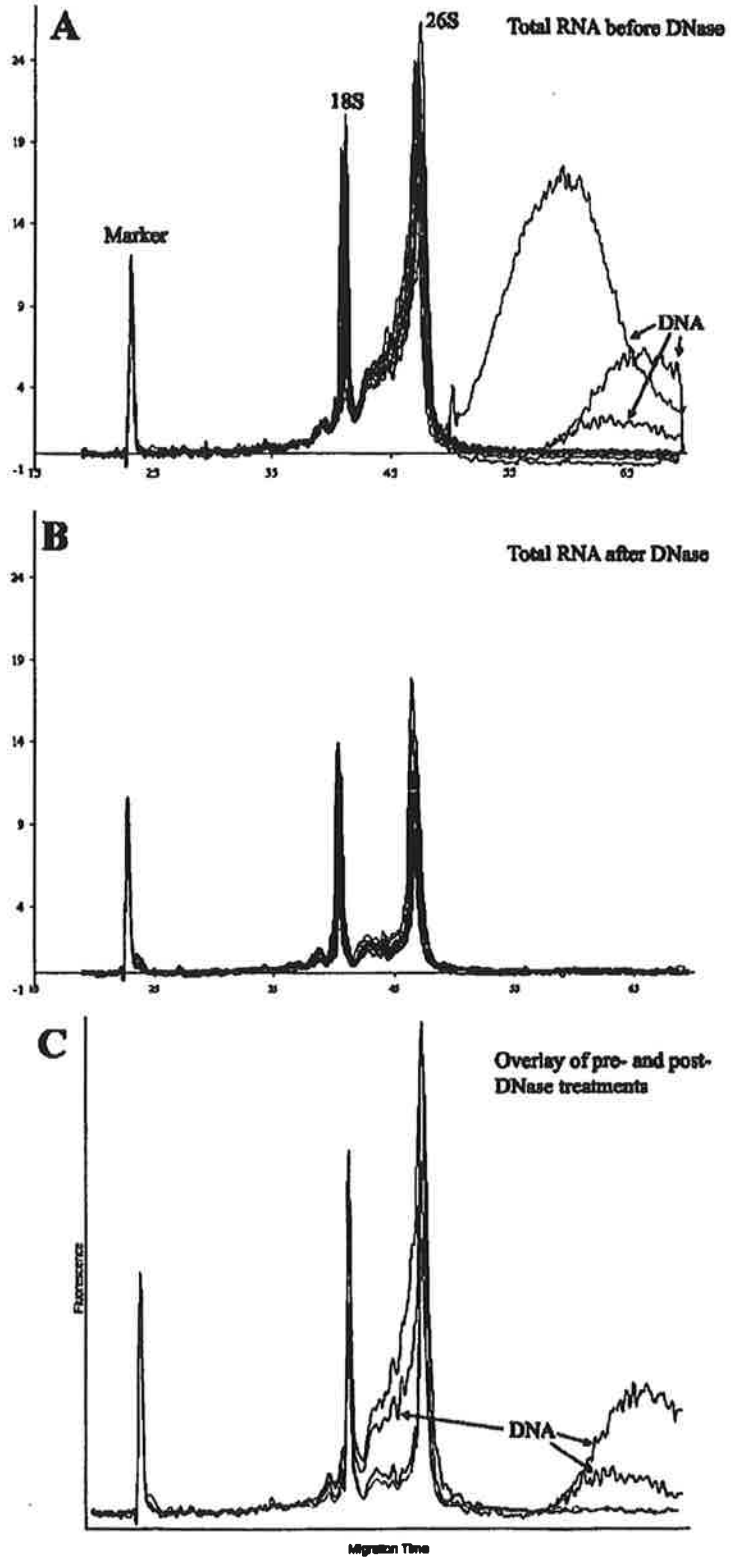

A
Total RNA before DNase
26S
18S
Marker
DNA
B
Total RNA after DNase
C
Overlay of pre- and post-DNase treatments
DNA

Figure 3

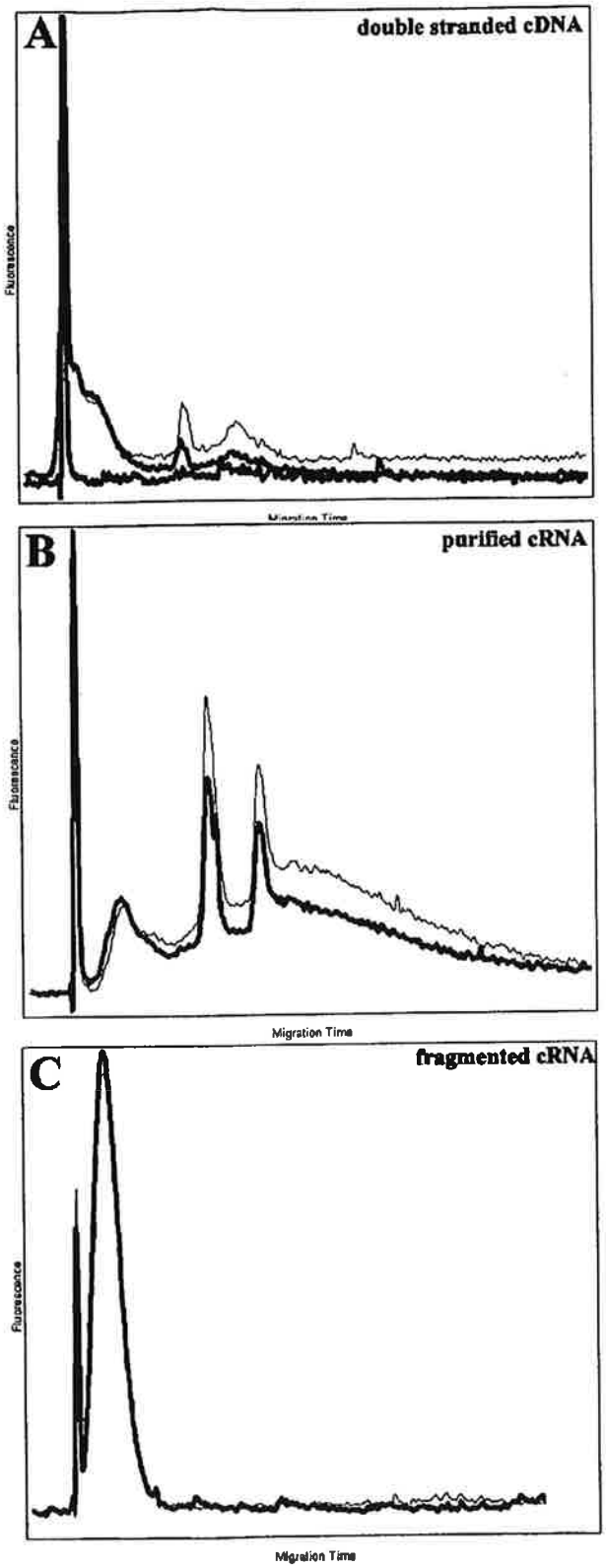

A
double stranded cDNA
Fluorescence
B
purified cRNA
Fluorescence
Migration Time
C
fragmented cRNA
Fluorescence
Migration Time

Figure 4

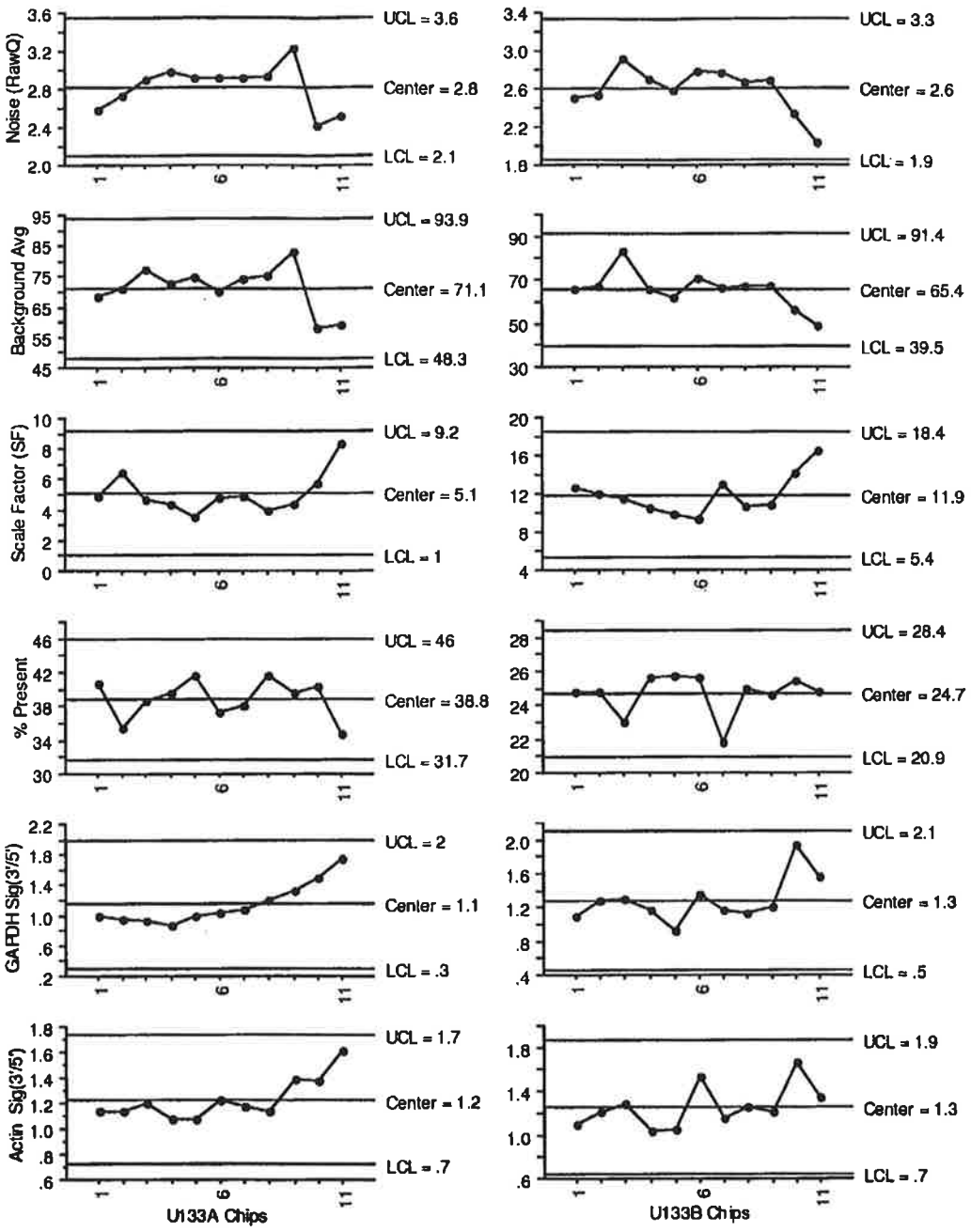

Noise (RawQ)
UCL = 3.6
Center = 2.8
LCL = 2.1
UCL = 3.3
Center = 2.6
LCL = 1.9
Background Avg
UCL = 93.9
Center = 71.1
LCL = 48.3
UCL = 91.4
Center = 65.4
LCL = 39.5
Scale Factor (SF)
UCL = 9.2
Center = 5.1
LCL = 1
UCL = 18.4
Center = 11.9
LCL = 5.4
% Present
UCL = 46
Center = 38.8
LCL = 31.7
UCL = 28.4
Center = 24.7
LCL = 20.9
GAPDH Sig(3'/5')
UCL = 2
Center = 1.1
LCL = .3
UCL = 2.1
Center = 1.3
LCL = .5
Actin Sig(3'/5')
UCL = 1.7
Center = 1.2
LCL = .7
UCL = 1.9
Center = 1.3
LCL = .7
U133A Chips
U133B Chips

Figure 5

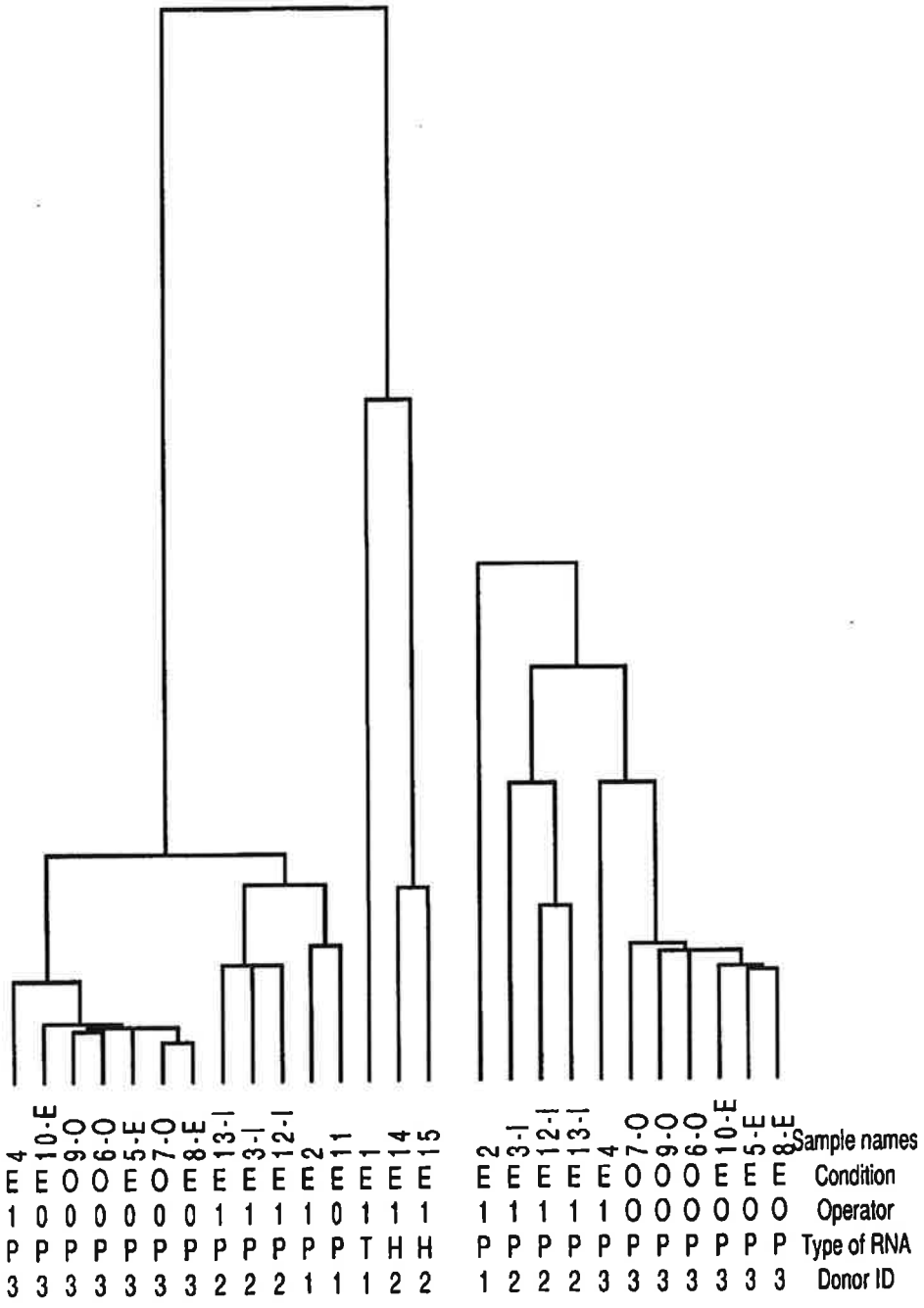

E 4
E 10-E
O 9-O
O 6-O
E 5-E
O 7-O
E 8-E
E 13-I
E 3-I
E 12-I
E 2
E 11
E 1
E 14
E 15
E 2
E 3-I
E 12-I
E 13-I
E 4
O 7-O
O 9-O
O 6-O
E 10-E
E 5-E
E 8-E
Sample names
E E O O E O E E E E E E E E E E E O O O E E E Condition
1 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0 Operator
P P P P P P P P P P P P P T H H P P P P P P P Type of RNA
3 3 3 3 3 3 3 2 2 2 1 1 1 2 2 1 2 2 2 3 3 3 3 3 3 3 Donor ID

Figure 6

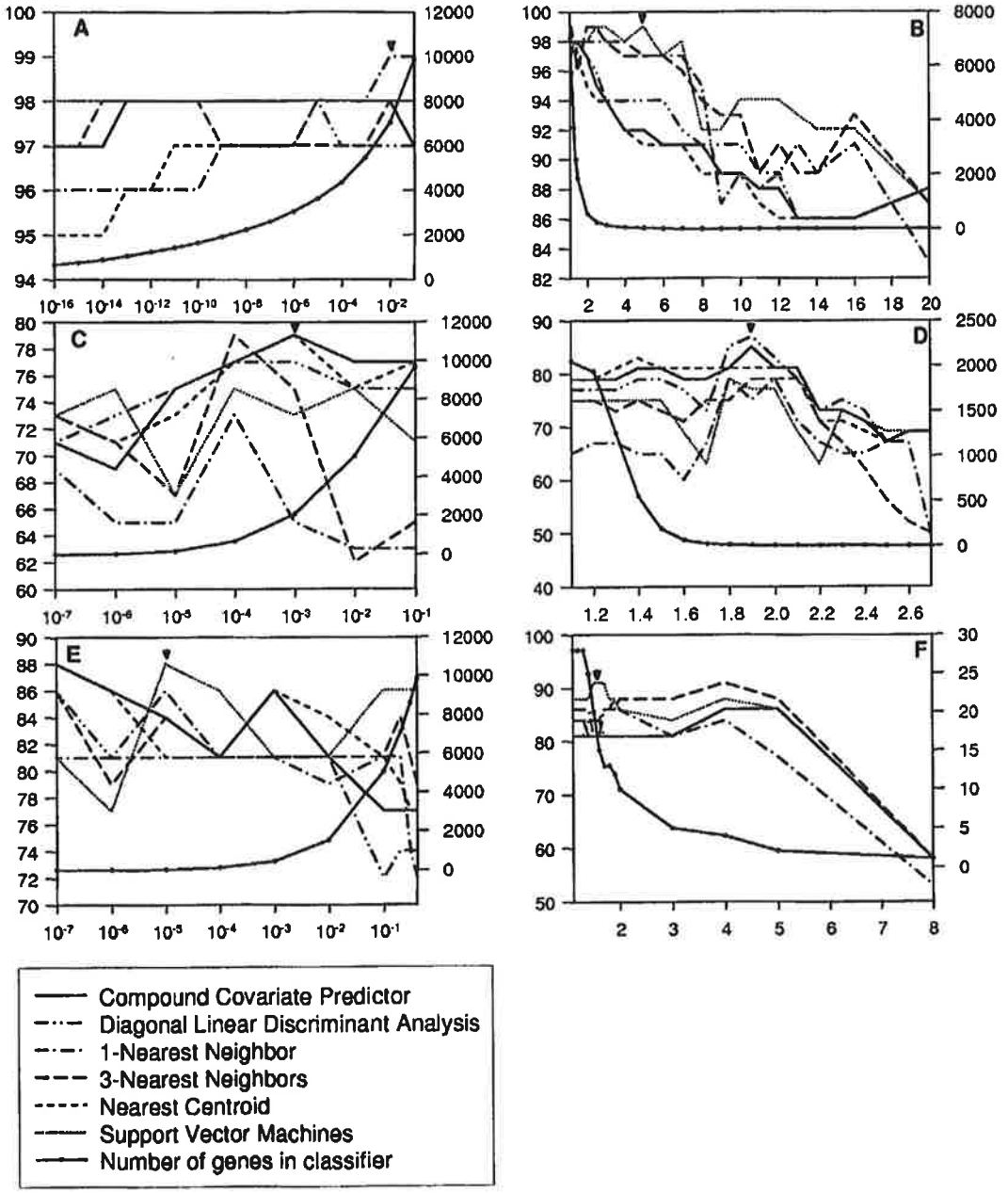

A
B
C
D
E
F
Compound Covariate Predictor
Diagonal Linear Discriminant Analysis
1-Nearest Neighbor
3-Nearest Neighbors
Nearest Centroid
Support Vector Machines
Number of genes in classifier

Figure 7

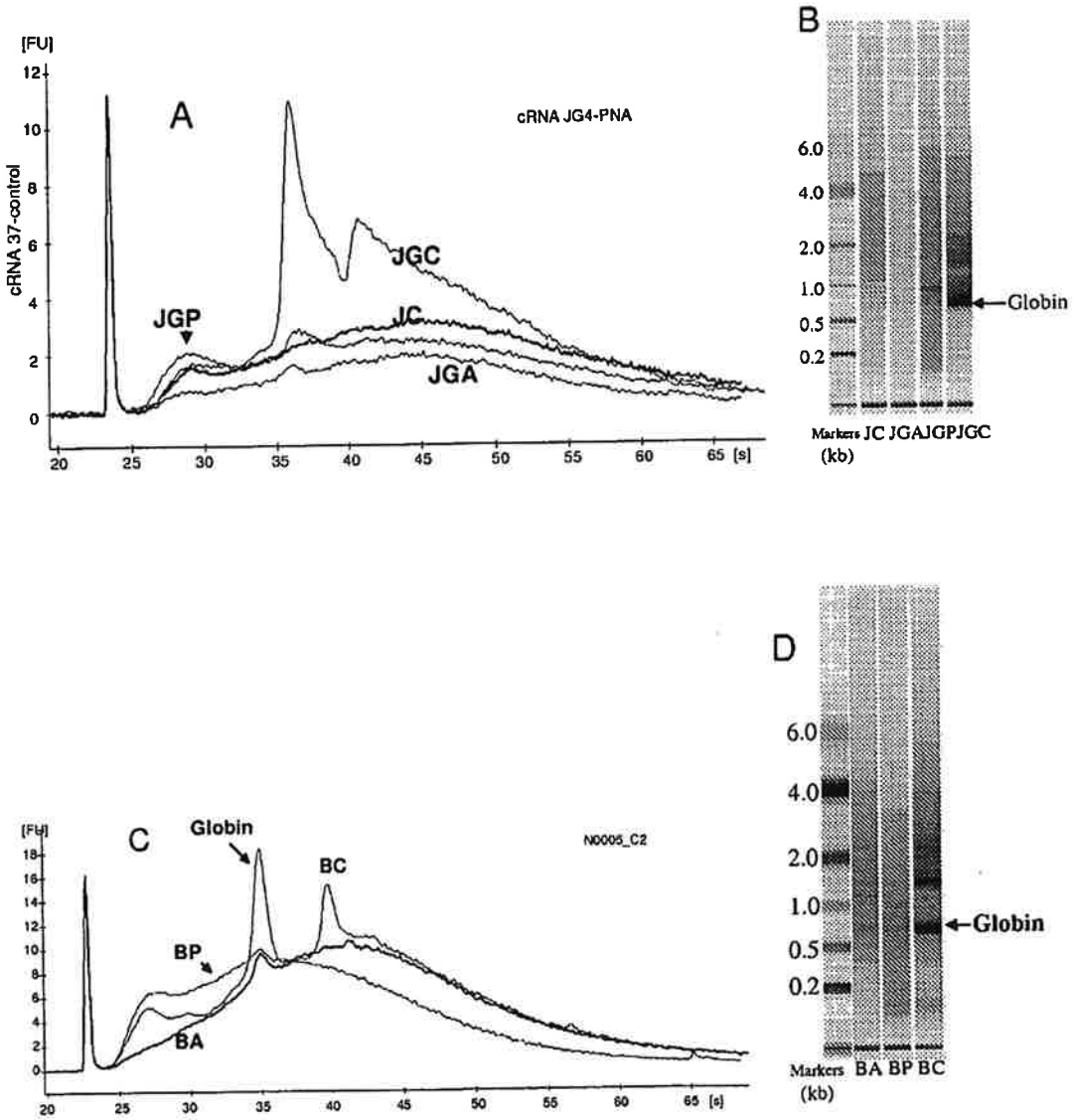

A
cRNA JG4-PNA
[FU]
cRNA 37-control
JGC
JC
JGA
JGP
20 25 30 35 40 45 50 55 60 65 [s]
B
6.0
4.0
2.0
1.0
0.5
0.2
Globin
Markers JC JGAJGPJGC
(kb)
C
Globin
BC
BP
BA
N0005_C2
D
Globin
Markers BA BP BC
(kb)

Figure 8

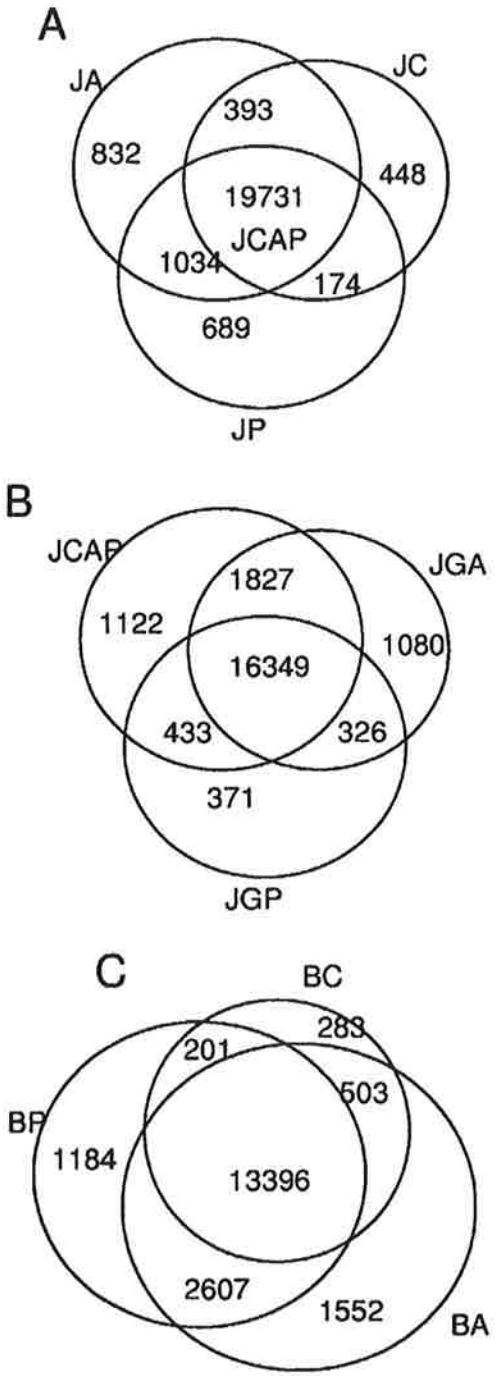
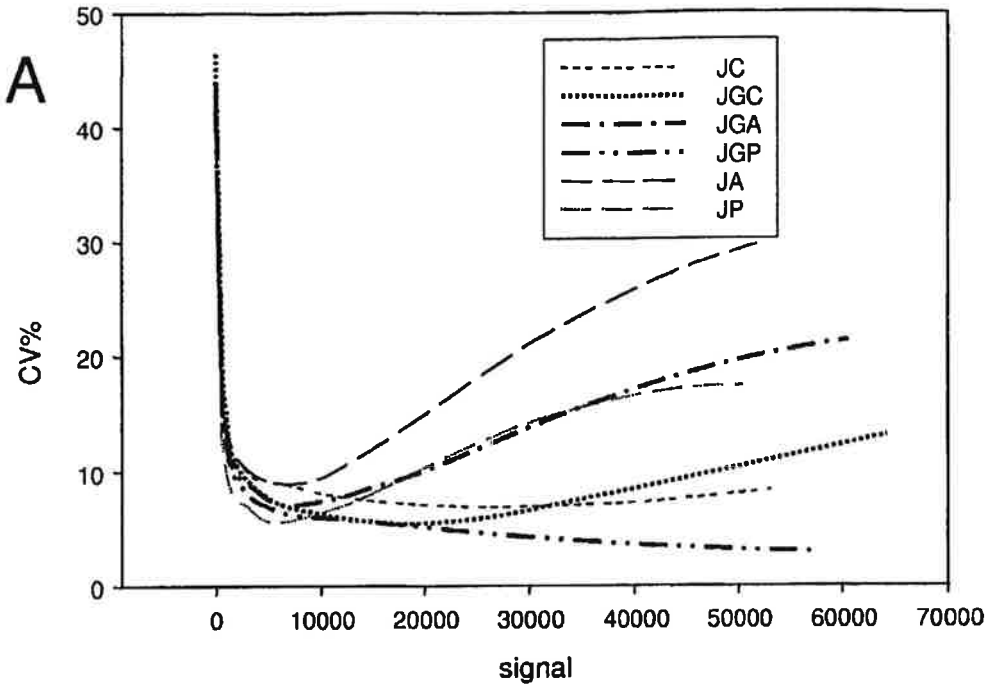

A
JA
JC
393
832
448
19731
JCAP
1034
174
689
JP
B
JCAP
JGA
1827
1122
1080
16349
433
326
371
JGP
C
BC
283
201
503
BP
1184
13396
2607
1552
BA

Figure 9



Paxgene Loess 2 degree freedom_scaling

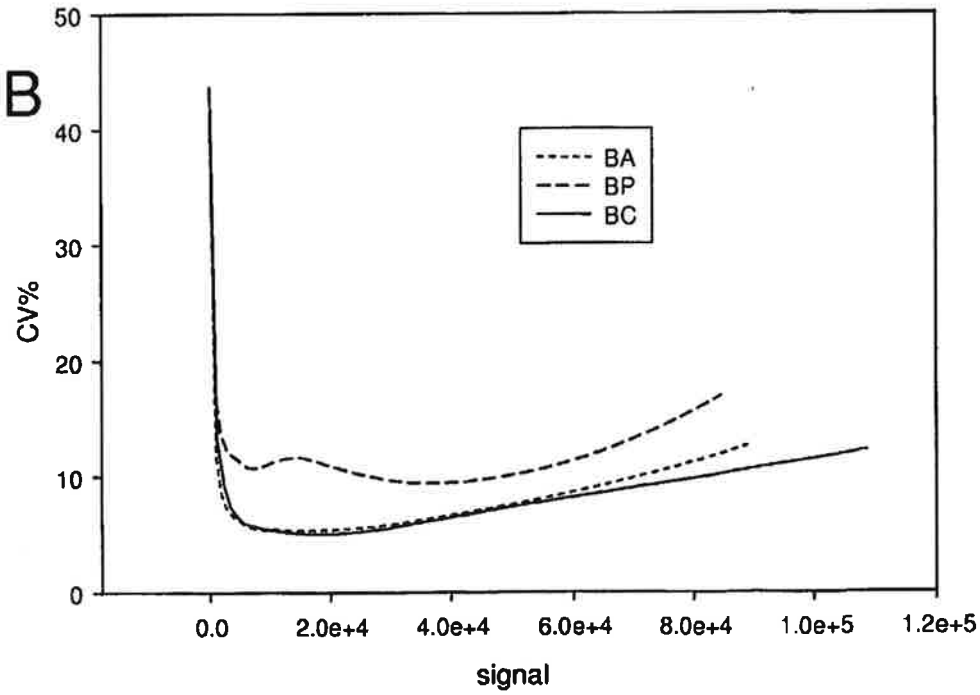


Figure 10

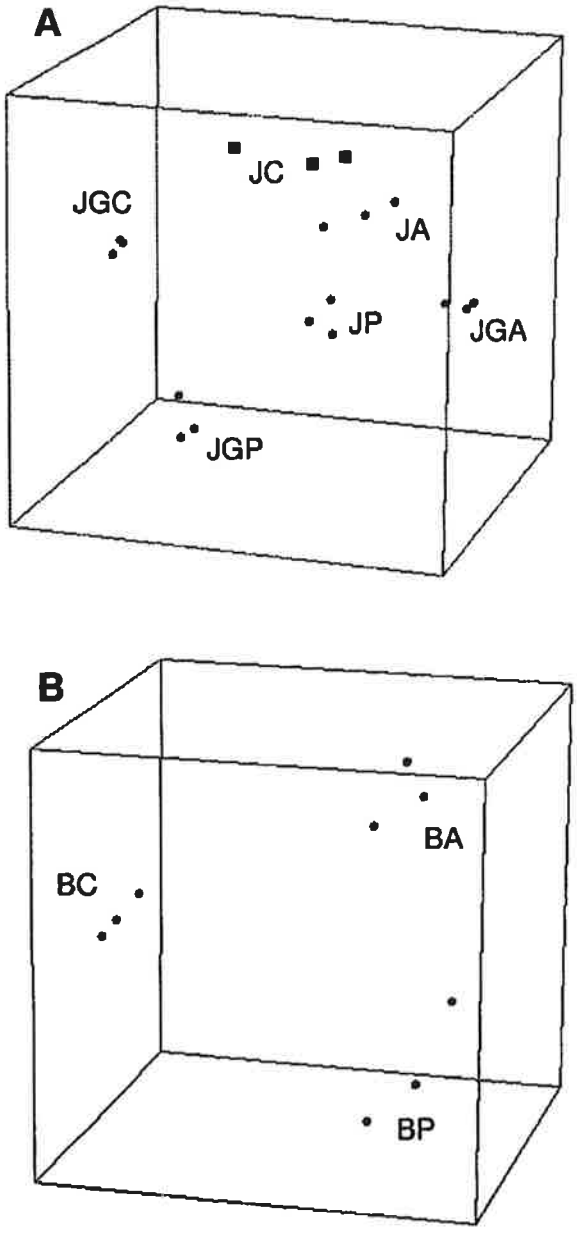


Figure 11

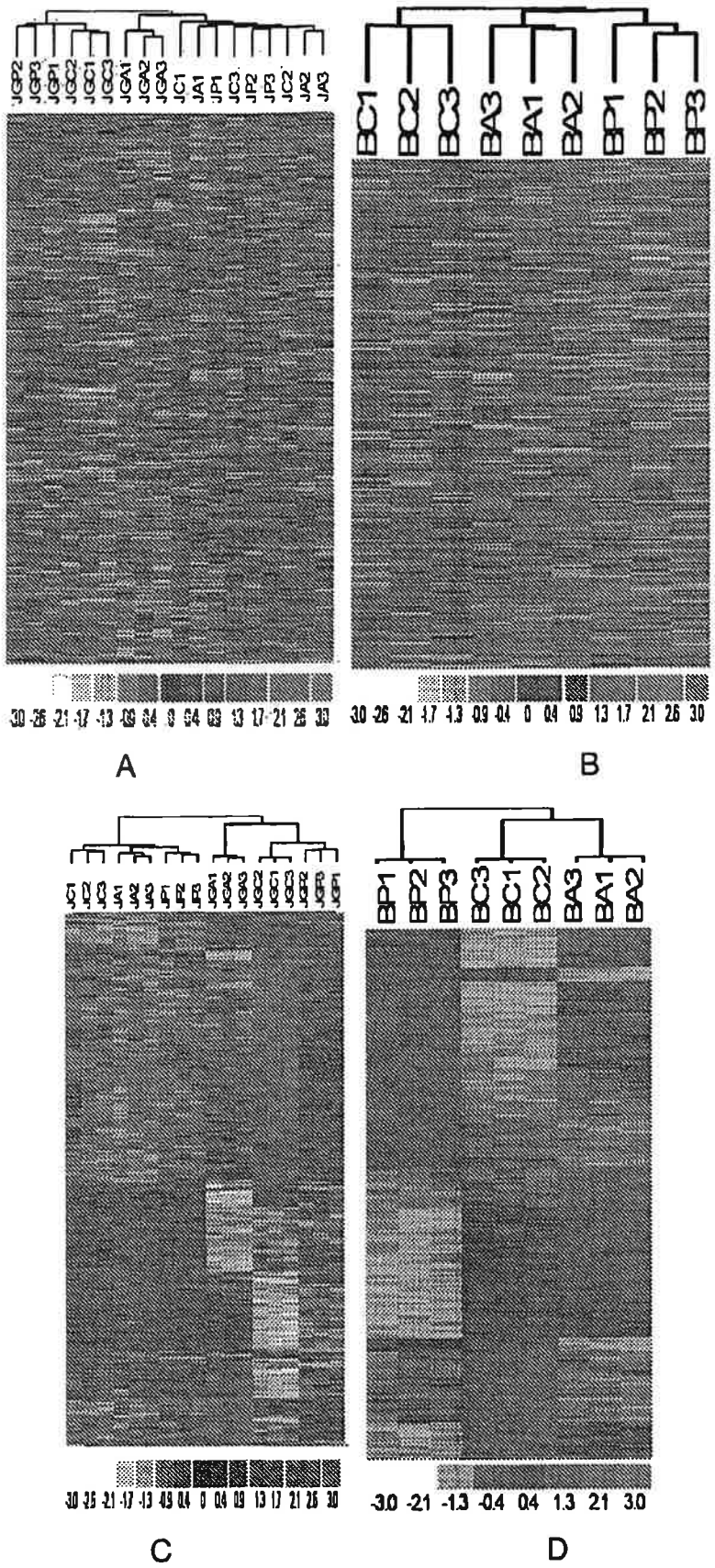

BC1
BC2
BC3
BA3
BA1
BA2
BP1
BP2
BP3
A
B
BP1
BP2
BP3
BC3
BC1
BC2
BA3
BA1
BA2
-3.0 -2.1 -1.3 -0.4 0.4 1.3 2.1 3.0
C
D

Figure 12

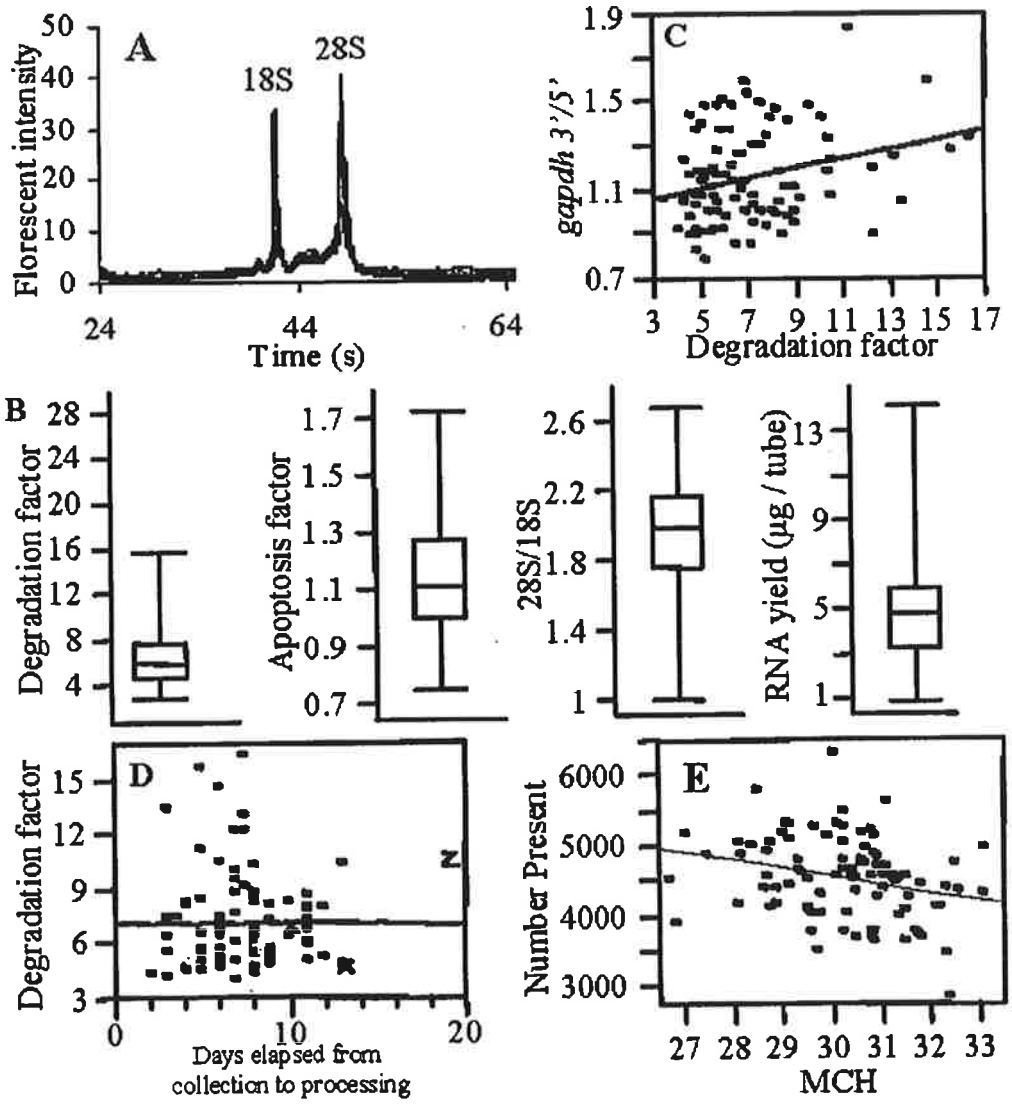

A
18S
28S
Florescent intensity
Time (s)
C
gapdh 3'/5'
Degradation factor
B
Degradation factor
Apoptosis factor
28S/18S
RNA yield (µg / tube)
D
Degradation factor
Days elapsed from collection to processing
E
Number Present
MCH

Figure 13

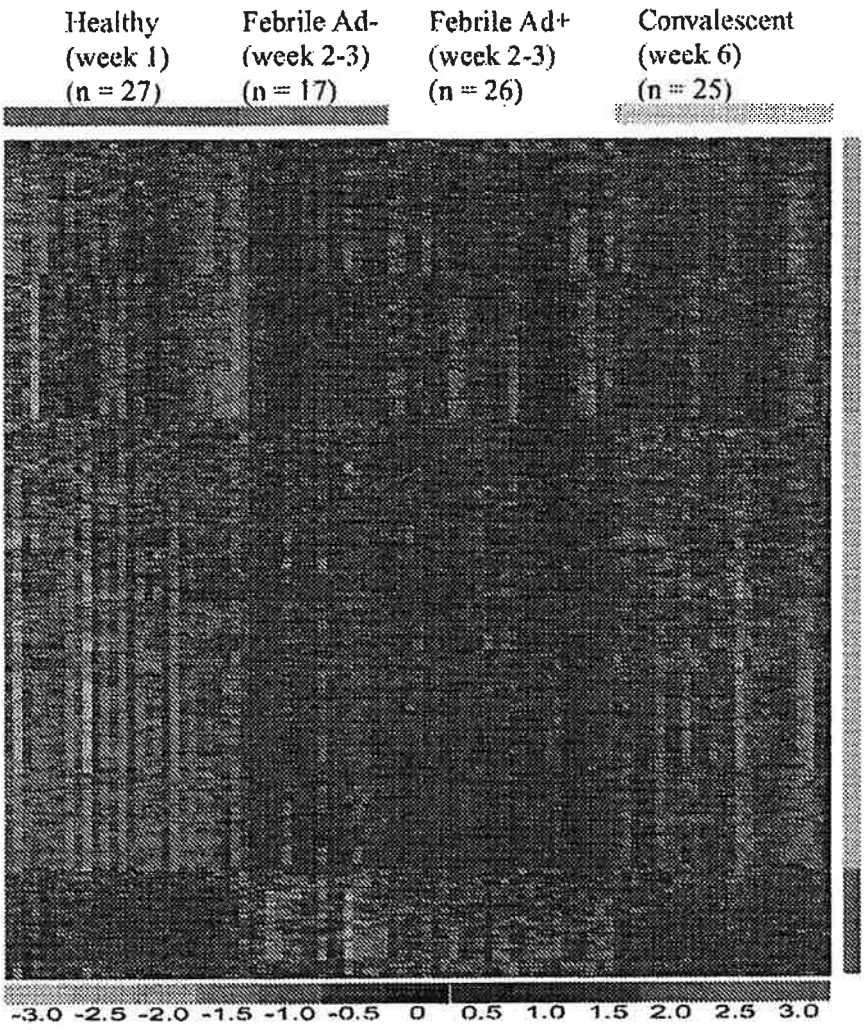

Healthy
(week 1)
(n = 27)
Febrile Ad-
(week 2-3)
(n = 17)
Febrile Ad+
(week 2-3)
(n = 26)
Convalescent
(week 6)
(n = 25)
-3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0 0.5 1.0 1.5 2.0 2.5 3.0

Figure 14

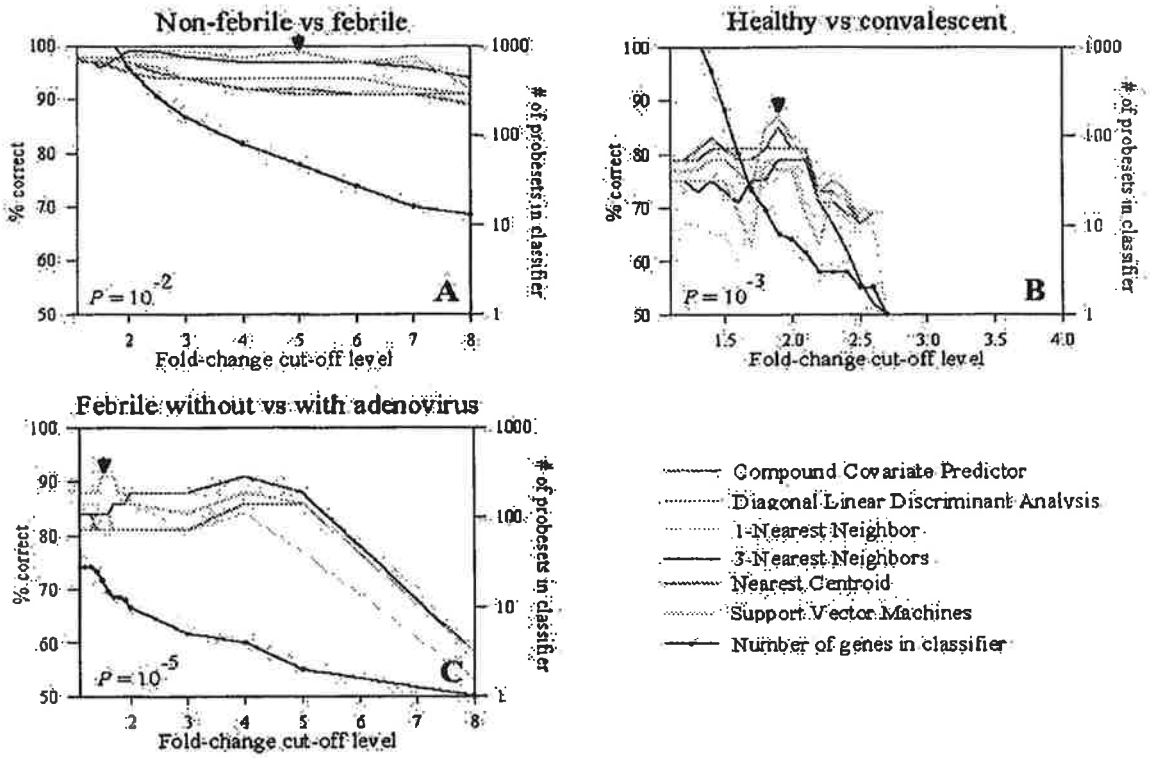

Non-febrile vs febrile
% correct
# of probesets in classifier
Fold-change cut-off level
P = 10^-2
A
Healthy vs convalescent
P = 10^-3
B
Febrile without vs with adenovirus
P = 10^-5
C
Compound Covariate Predictor
Diagonal Linear Discriminant Analysis
1-Nearest Neighbor
3-Nearest Neighbors
Nearest Centroid
Support Vector Machines
Number of genes in classifier

Figure 15

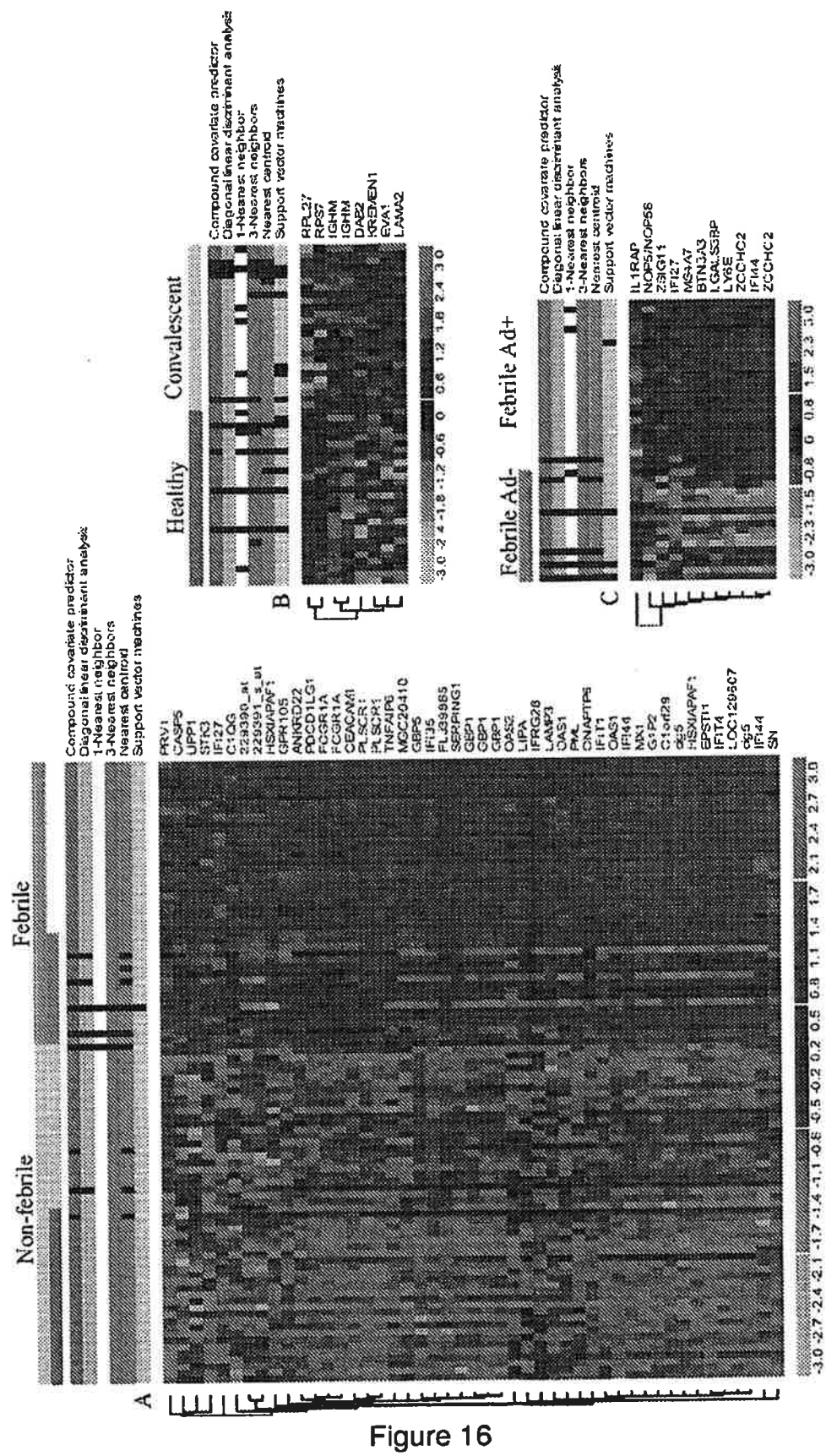

Non-febrile
Febrile
Compound covariate predictor
Diagonal linear discriminant analysis
1-Nearest neighbor
3-Nearest neighbors
Nearest centroid
Support vector machines
A
PRV1
CASP5
UPP1
STK3
IFI27
C1QG
229390_at
229391_s_at
HSXIAPAF1
GPR105
ANKRD22
PDCD1LG1
FCGR1A
CEACAM8
PLSCR1
TNFAIP6
MGC20410
GBP5
IFI35
FLJ39985
SERPING1
GBP1
GBP1
GBP1
OASL
LIPA
IFRG28
LAMP3
OAS1
PML
DNAPTP6
IFIT1
OAS1
IFI44
NMI
G1P2
C1orf29
gs5
HSXIAPAF1
EPSTI1
IFIT4
LOC129607
gs5
IFI44
SN
-3.0 -2.7 -2.4 -2.1 -1.7 -1.4 -1.1 -0.8 -0.5 -0.2 0.2 0.5 0.8 1.1 1.4 1.7 2.1 2.4 2.7 3.0
Healthy
Convalescent
Compound covariate predictor
Diagonal linear discriminant analysis
1-Nearest neighbor
3-Nearest neighbors
Nearest centroid
Support vector machines
B
RPL27
RPS7
IGHM
IGHM
DAB2
KREMEN1
EVA1
LAMA2
-3.0 -2.4 -1.8 -1.2 -0.6 0 0.6 1.2 1.8 2.4 3.0
Febrile Ad-
Febrile Ad+
Compound covariate predictor
Diagonal linear discriminant analysis
1-Nearest neighbor
3-Nearest neighbors
Nearest centroid
Support vector machines
C
IL1RAP
NOP5/NOP58
ZBIG11
IFI27
MGA7
BTN3A3
LGALS3BP
LY6E
ZCCHC2
IFI44
ZCCHC2
-3.0 -2.3 -1.5 -0.8 0 0.8 1.5 2.3 3.0

Figure 16