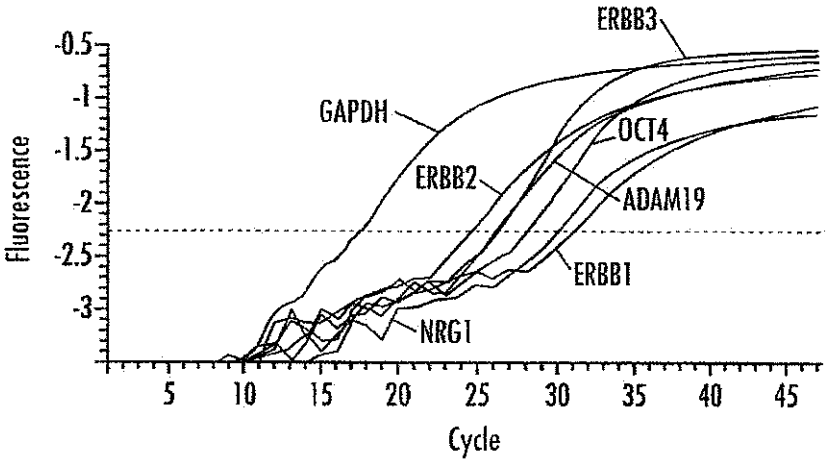


Fig. 1

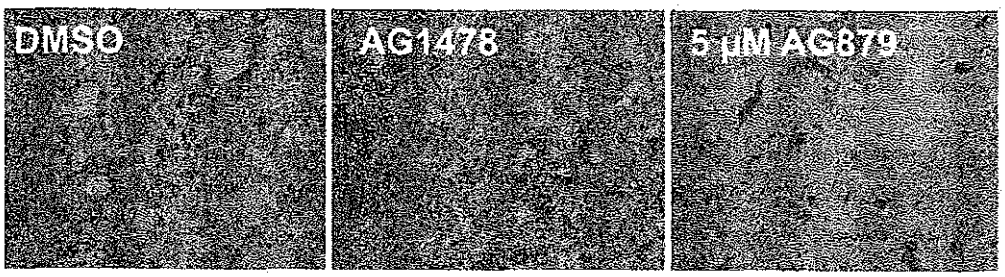


Fig. 2A Fig. 2B Fig. 2C

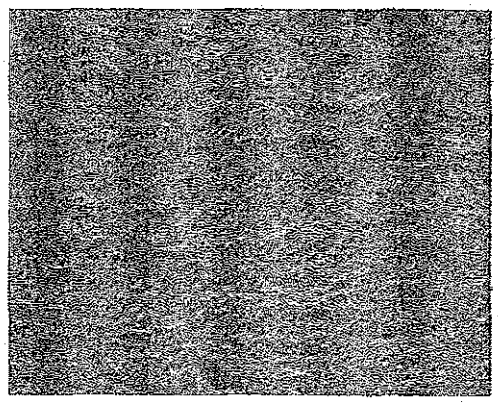


Fig. 3A

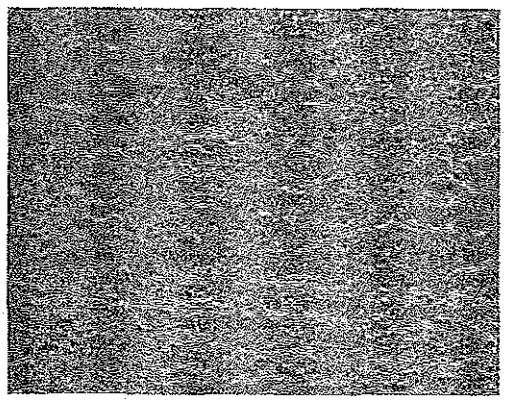


Fig. 3B

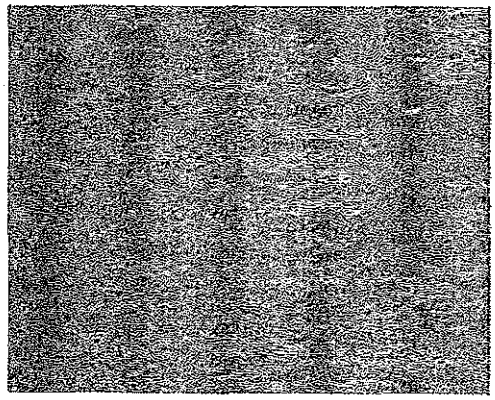


Fig. 3C

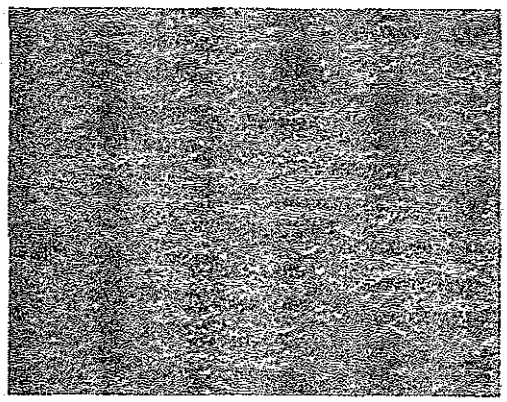


Fig. 3D

Mouse ES

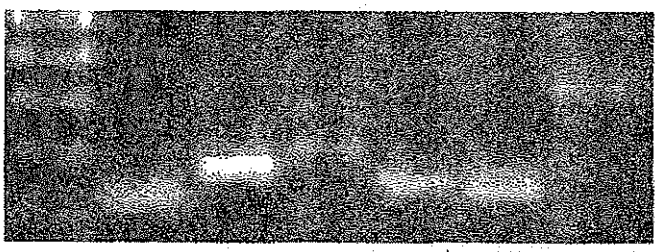


Fig. 4A

MEFs

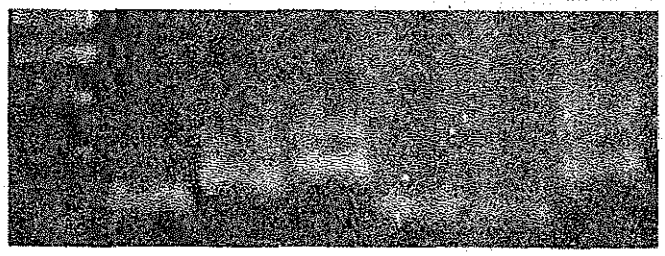


Fig. 4B

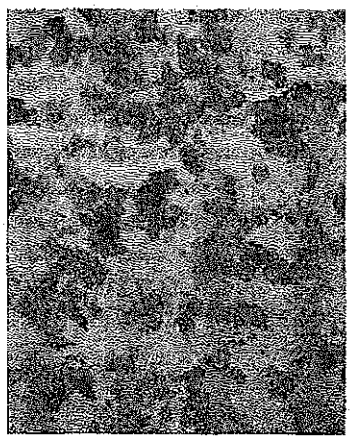


Fig. 5A



Fig. 5B

Fig. 5C

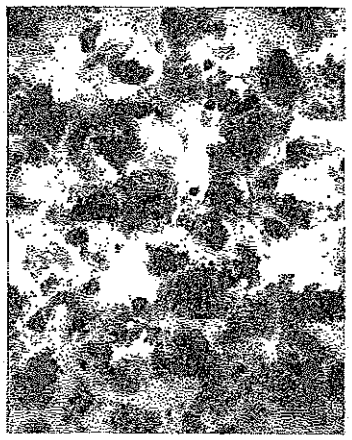


Fig. 5D

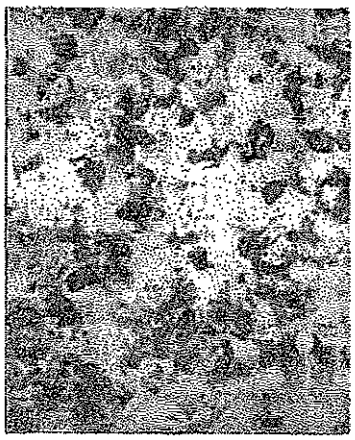


Fig. 5E

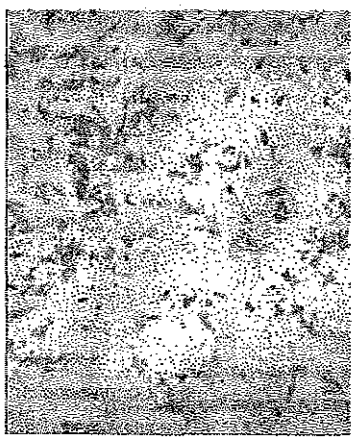


Fig. 5F

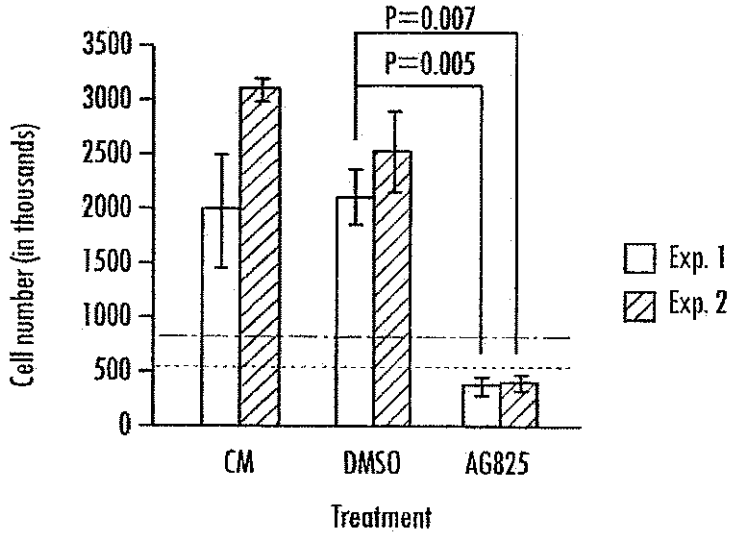

P=0.007
P=0.005
3500
3000
2500
2000
1500
1000
500
0
Cell number (in thousands)
CM
DMSO
AG825
Treatment
Exp. 1
Exp. 2

Fig. 6A

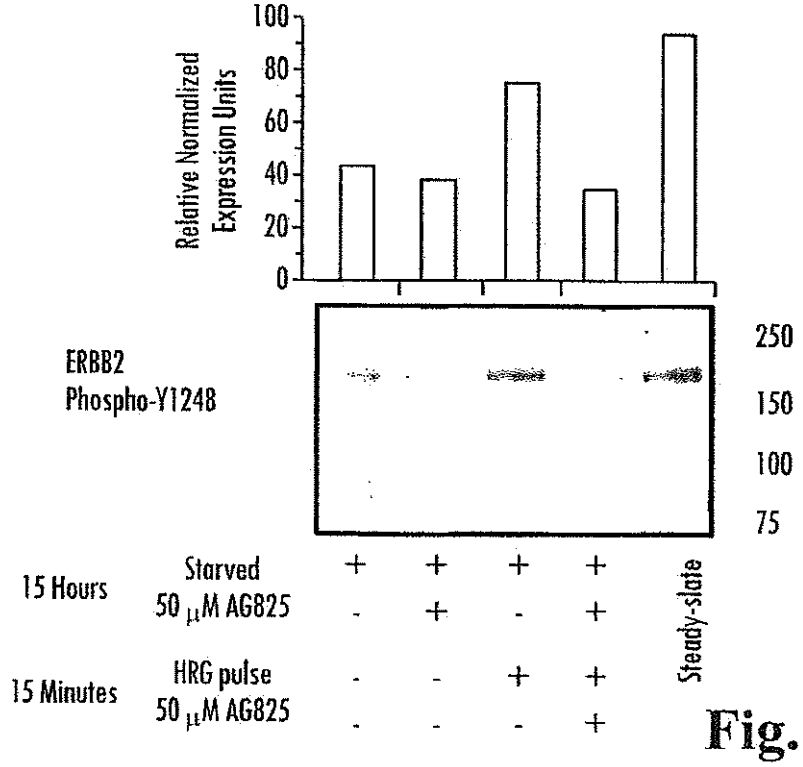

100
80
60
40
20
0
Relative Normalized Expression Units
ERBB2
Phospho-Y1248
250
150
100
75
15 Hours
Starved
50 μM AG825
15 Minutes
HRG pulse
50 μM AG825
Steady-state

Fig. 6B

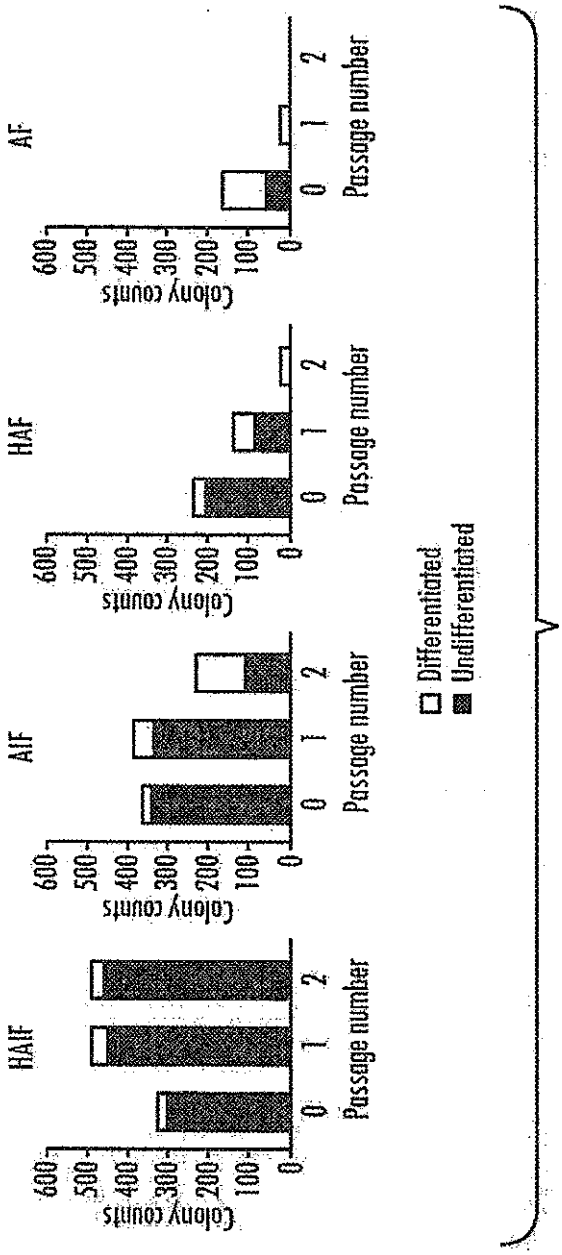

HAIF
AIF
HAF
AF
Colony counts
Passage number
0
1
2
100
200
300
400
500
600
Differentiated
Undifferentiated

Fig. 6C

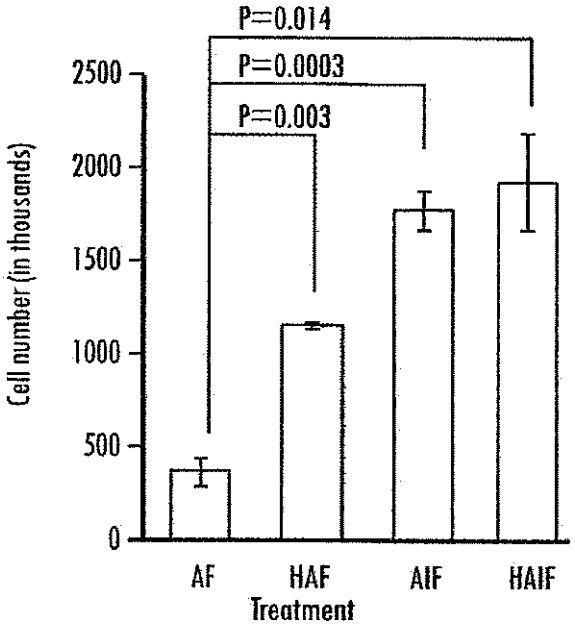

P=0.014
P=0.0003
P=0.003
2500
2000
1500
1000
500
0
Cell number (in thousands)
AF
HAF
AIF
HAIF
Treatment

Fig. 6D

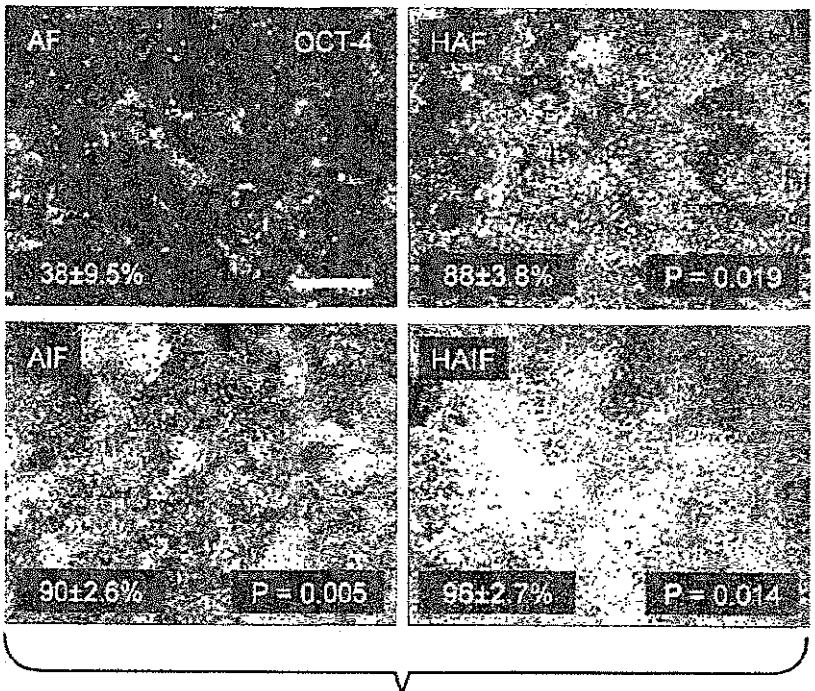

AF
OCT-4
38±9.5%
HAF
88±3.8%
P = 0.019
AIF
90±2.6%
P = 0.005
HAIF
96±2.7%
P = 0.014

Fig. 6E

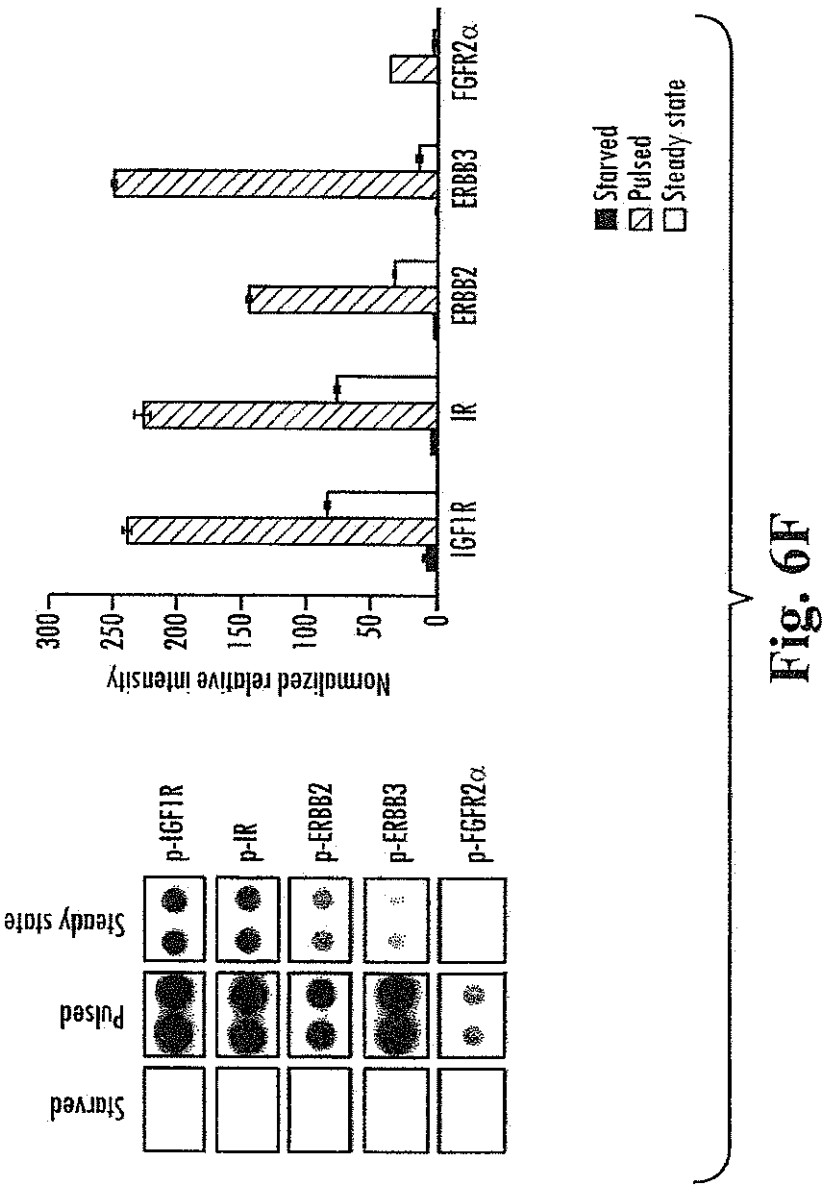

Starved
Pulsed
Steady state
p-IGF1R
p-IR
p-ERBB2
p-ERBB3
p-FGFR2α
Normalized relative intensity
300
250
200
150
100
50
0
IGF1R
IR
ERBB2
ERBB3
FGFR2α
Starved
Pulsed
Steady state

Fig. 6F

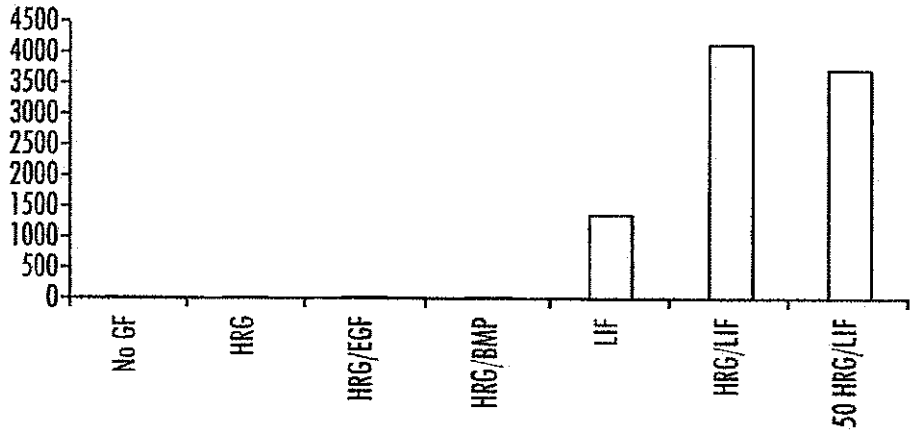


Fig. 7A

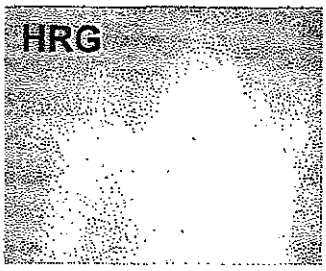


Fig. 7B

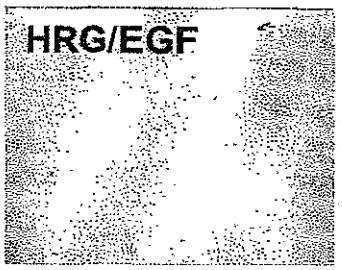


Fig. 7C

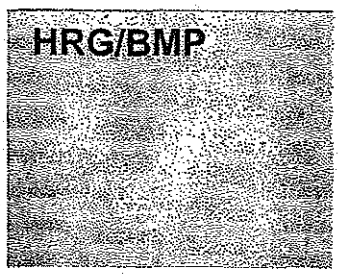


Fig. 7D



Fig. 7E



Fig. 7F

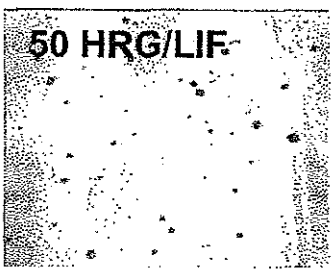


Fig. 7G

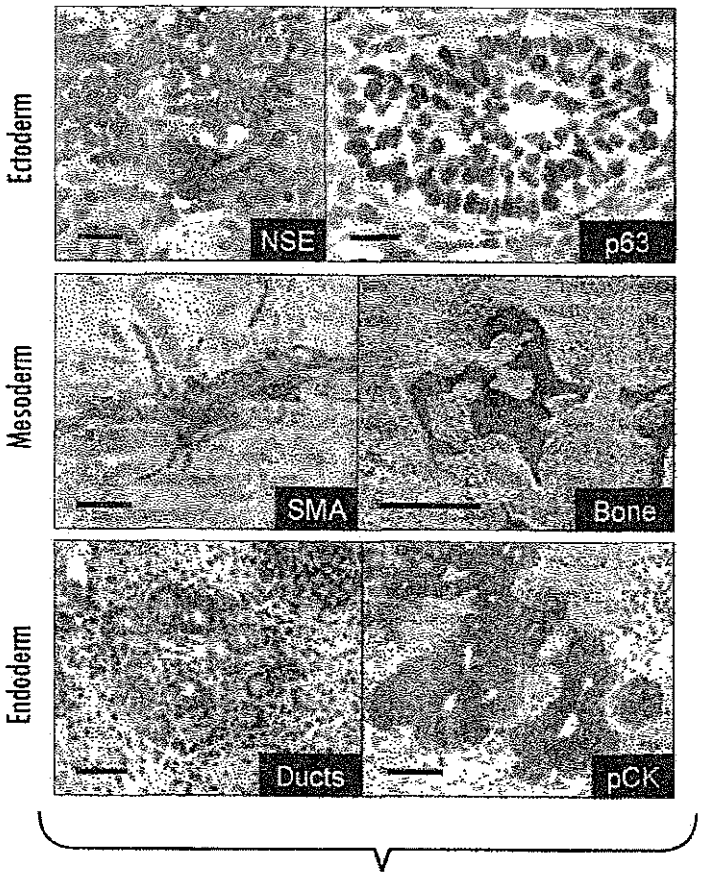

Ectoderm
NSE
p63
Mesoderm
SMA
Bone
Endoderm
Ducts
pCK

Fig. 8A

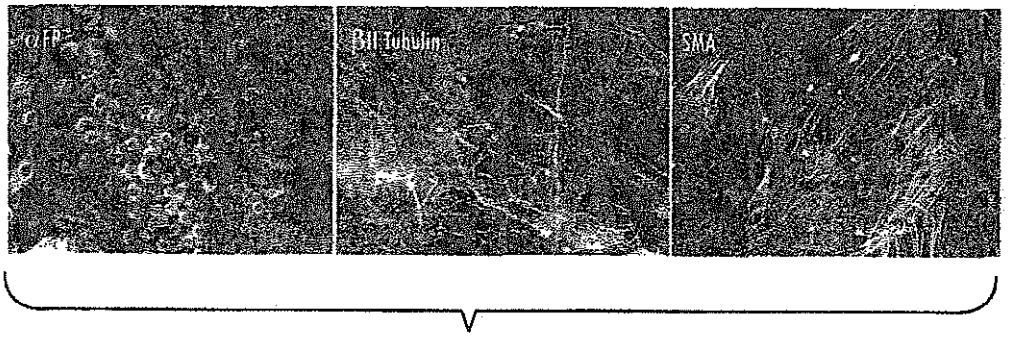

αFP
BIII Tubulin
SMA

Fig. 8B

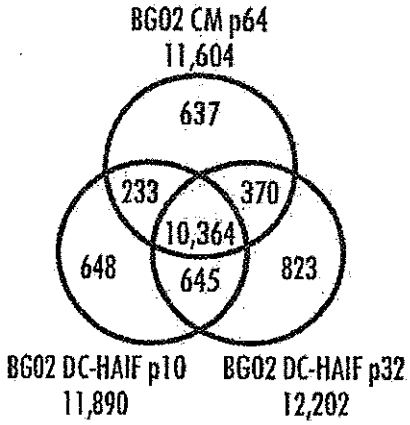


Fig. 8C

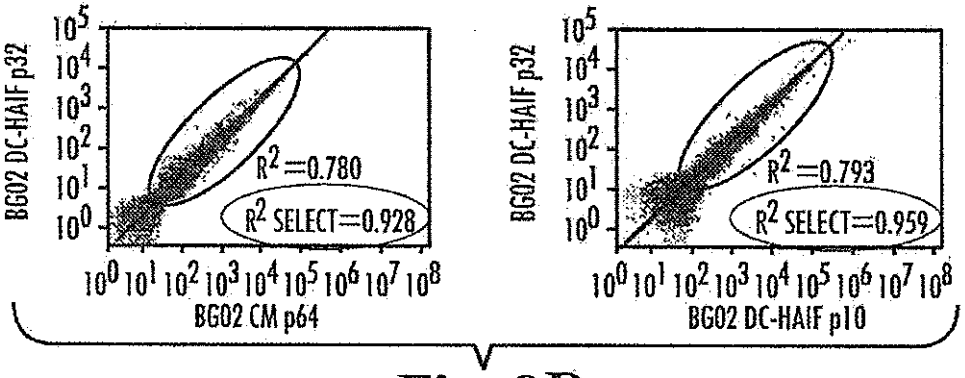


Fig. 8D

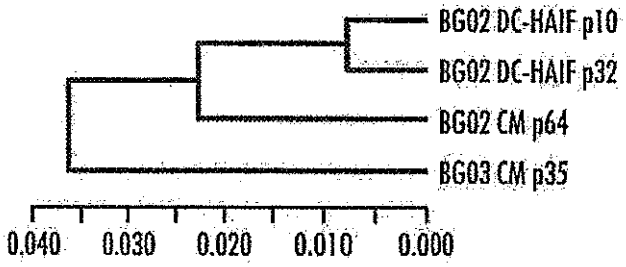


Fig. 8E

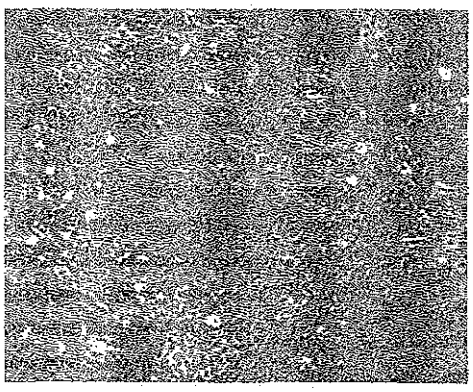


Fig. 9A

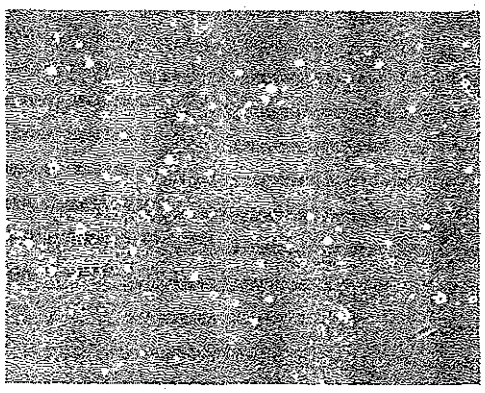


Fig. 9B

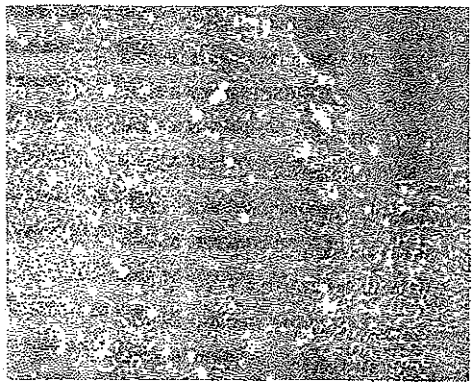


Fig. 9C

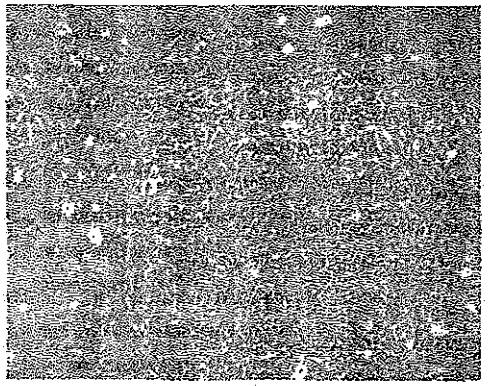


Fig. 9D

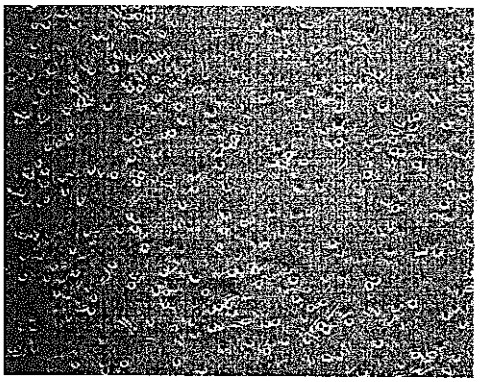


Fig. 10A

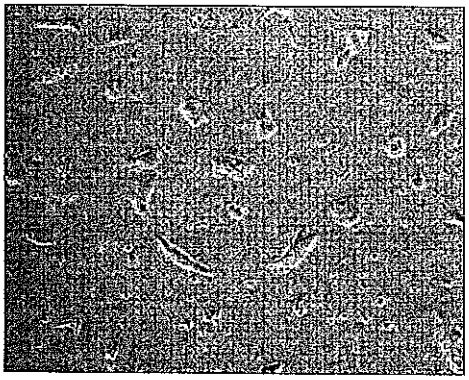


Fig. 10B

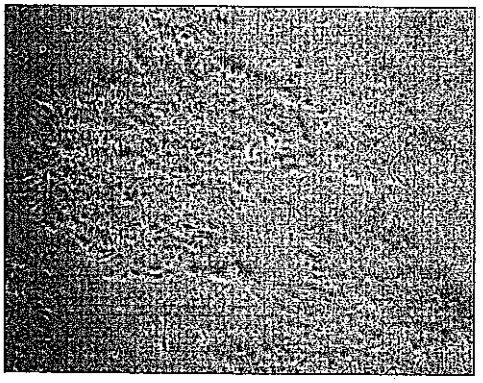


Fig. 10C

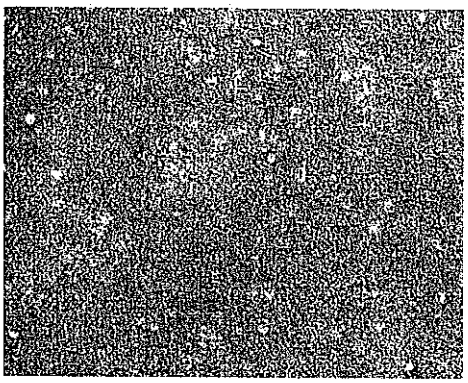


Fig. 10D

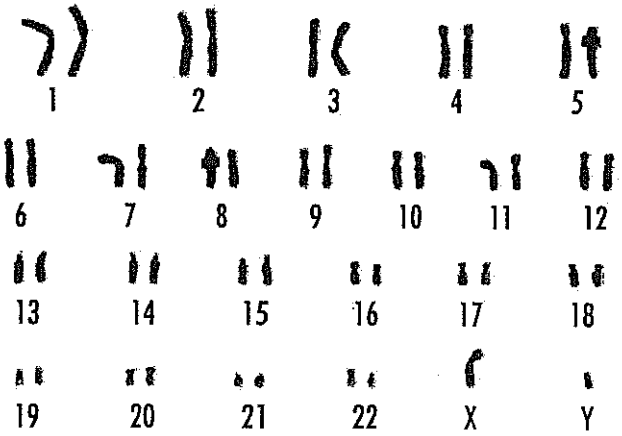


Fig. 10E

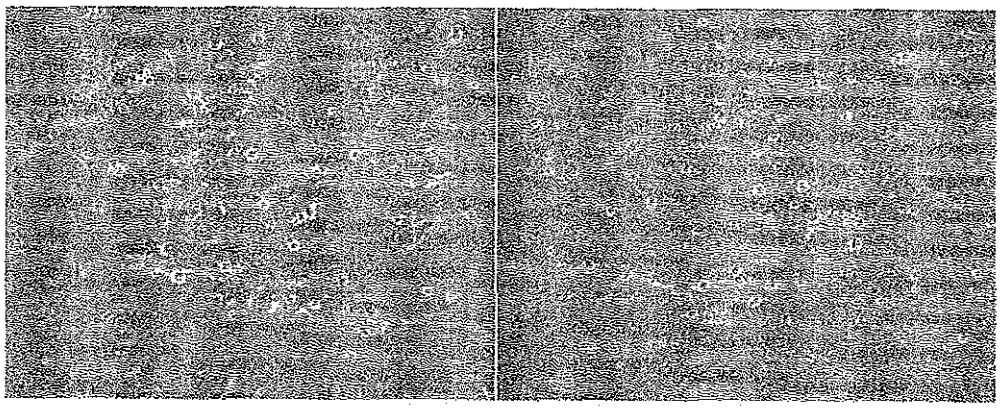


Fig. 11A

Fig. 11B

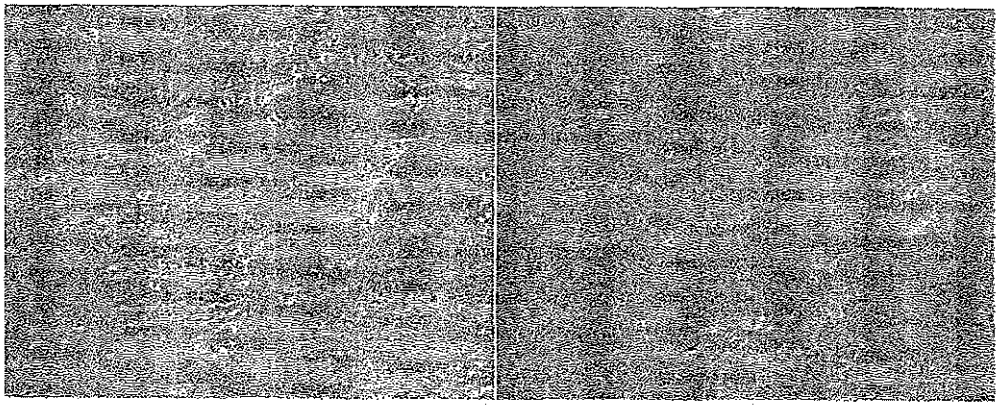


Fig. 11C

Fig. 11D

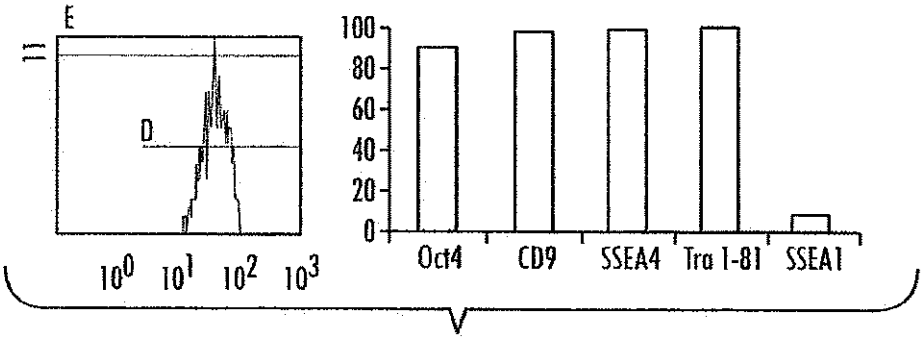


Fig. 12A

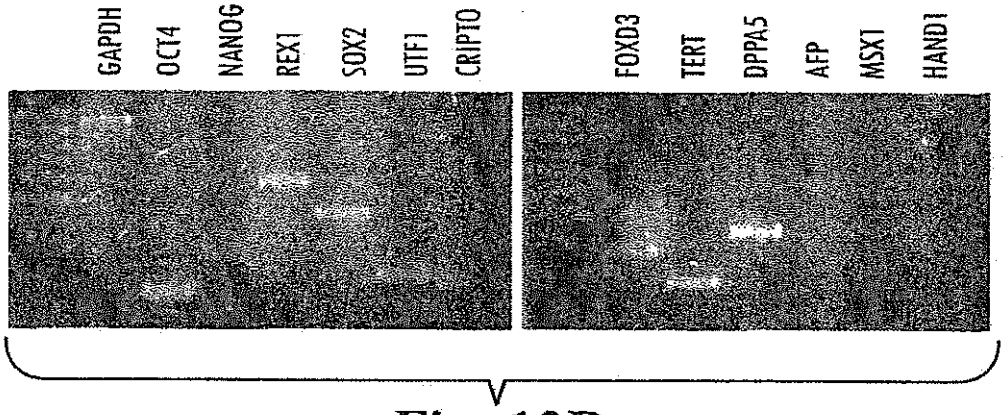


Fig. 12B

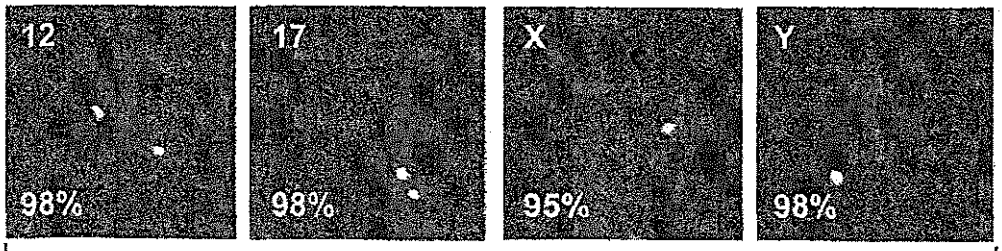


Fig. 12C

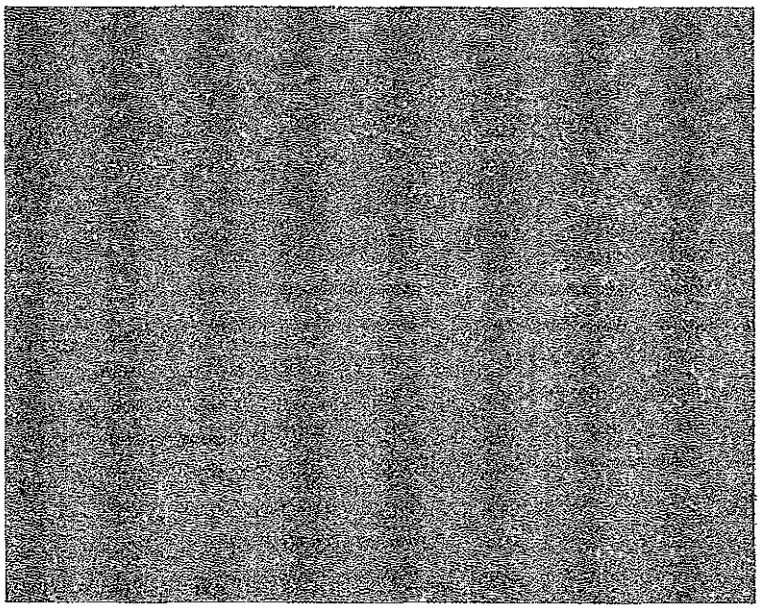


Fig. 13A

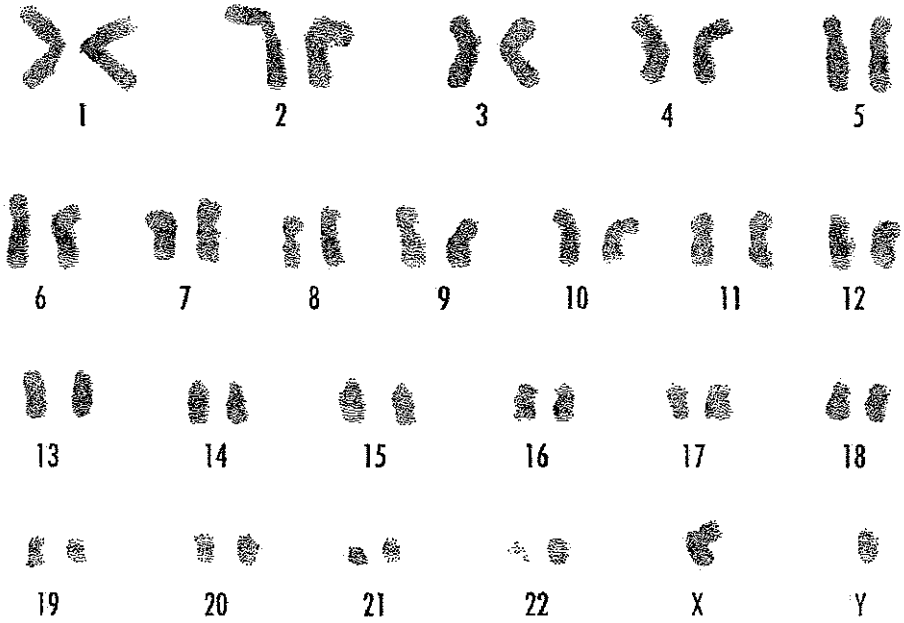


Fig. 13B

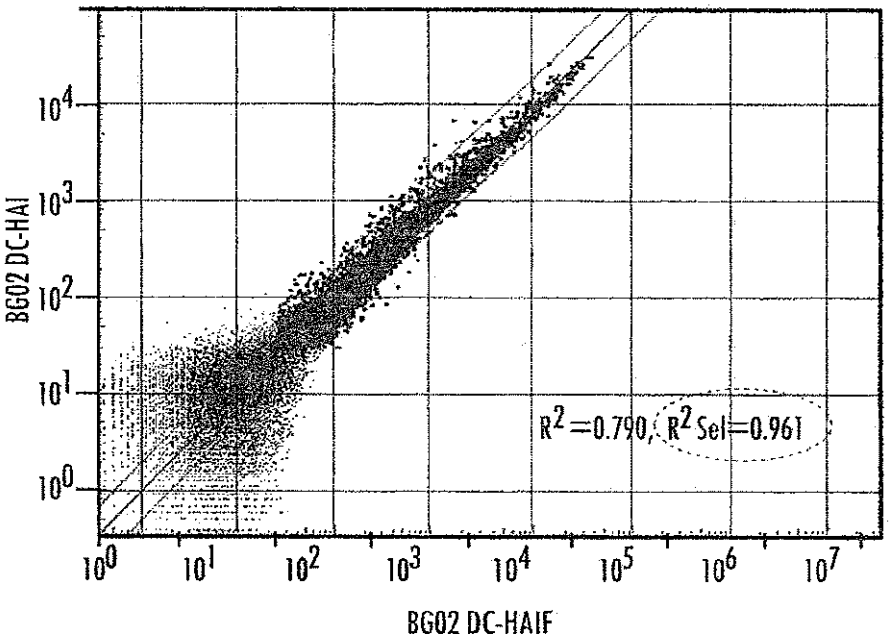

BG02 DC-HAI
10^4
10^3
10^2
10^1
10^0
R^2=0.790, R^2Sel=0.961
10^0 10^1 10^2 10^3 10^4 10^5 10^6 10^7
BG02 DC-HAIF

Fig. 14

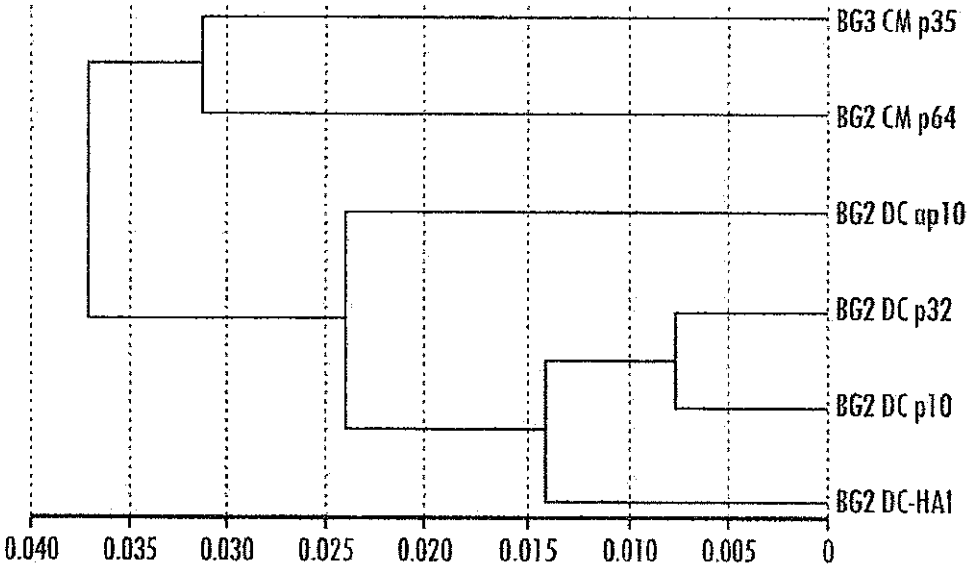

BG3 CM p35
BG2 CM p64
BG2 DC ap10
BG2 DC p32
BG2 DC p10
BG2 DC-HA1
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0

Fig. 15

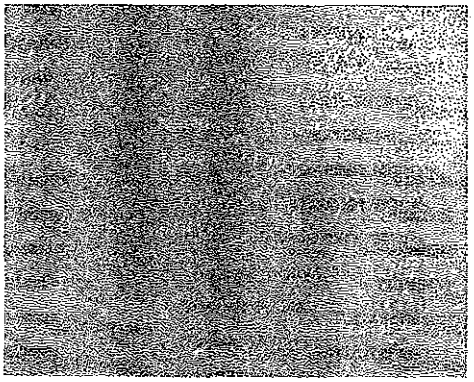


Fig. 16A



Fig. 16B

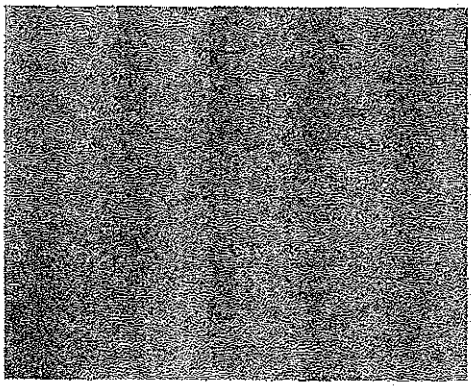


Fig. 16C

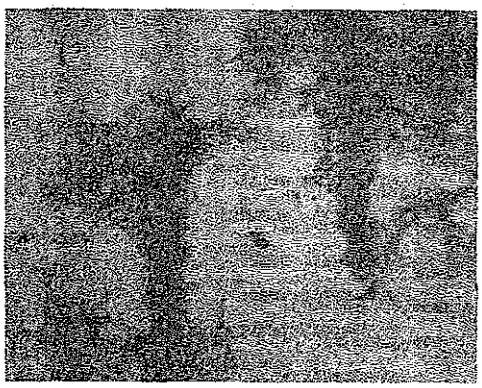


Fig. 16D

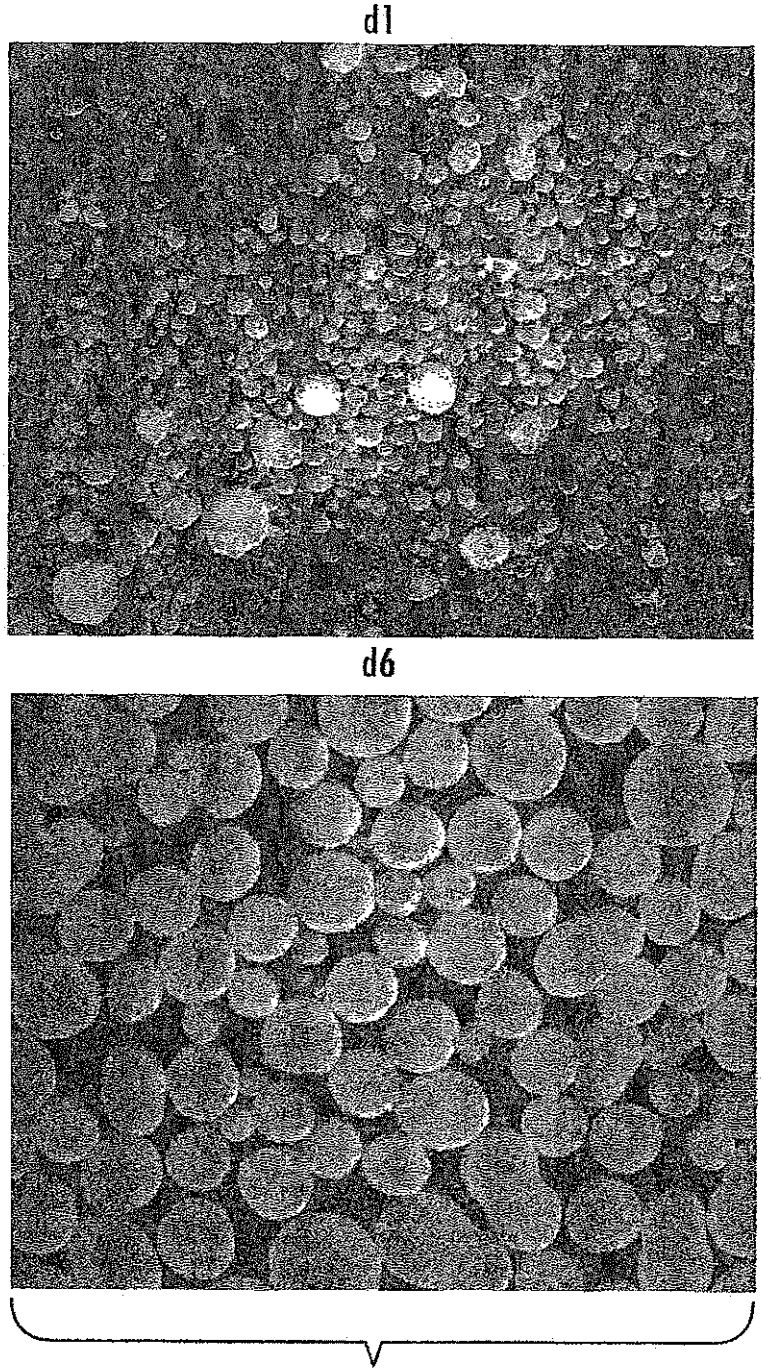


Fig. 17

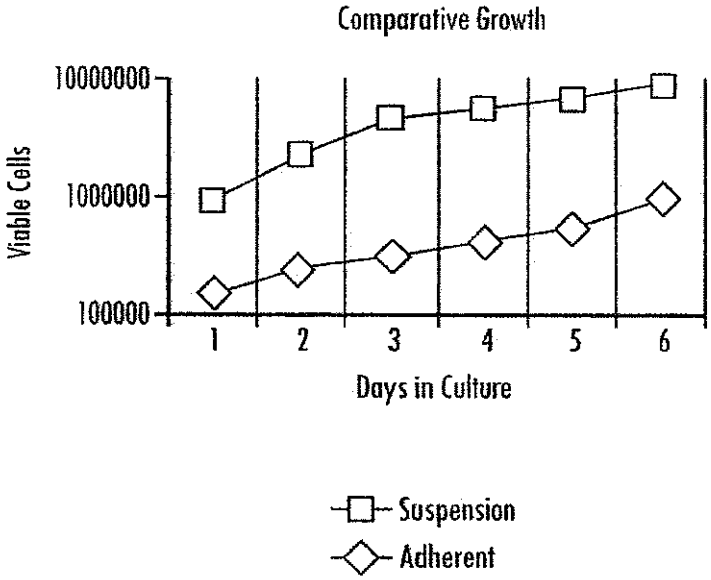

Comparative Growth
10000000
1000000
100000
Viable Cells
1
2
3
4
5
6
Days in Culture
Suspension
Adherent

Fig. 18

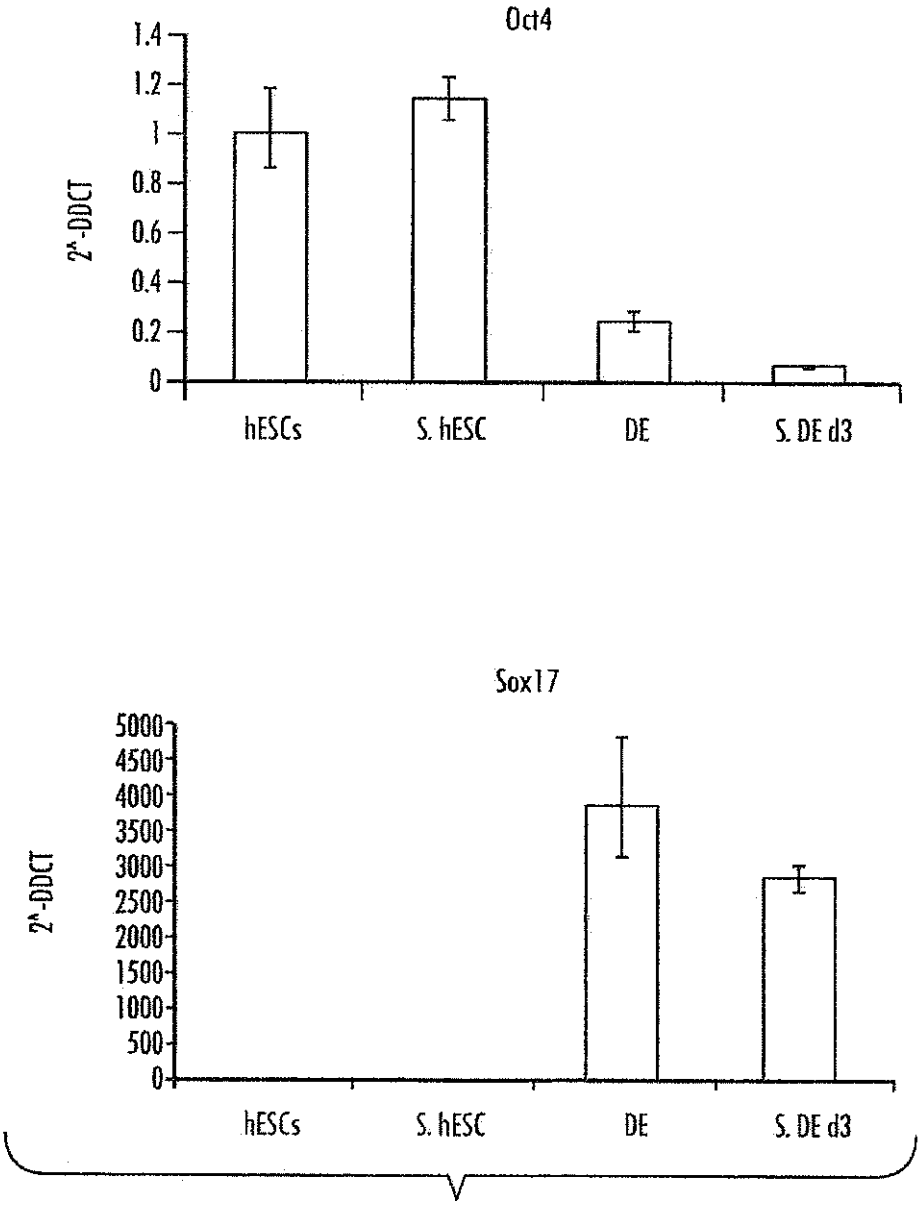

Oct4
2^-DDCT
1.4
1.2
1
0.8
0.6
0.4
0.2
0
hESCs
S. hESC
DE
S. DE d3
Sox17
2^-DDCT
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
hESCs
S. hESC
DE
S. DE d3

Fig. 19

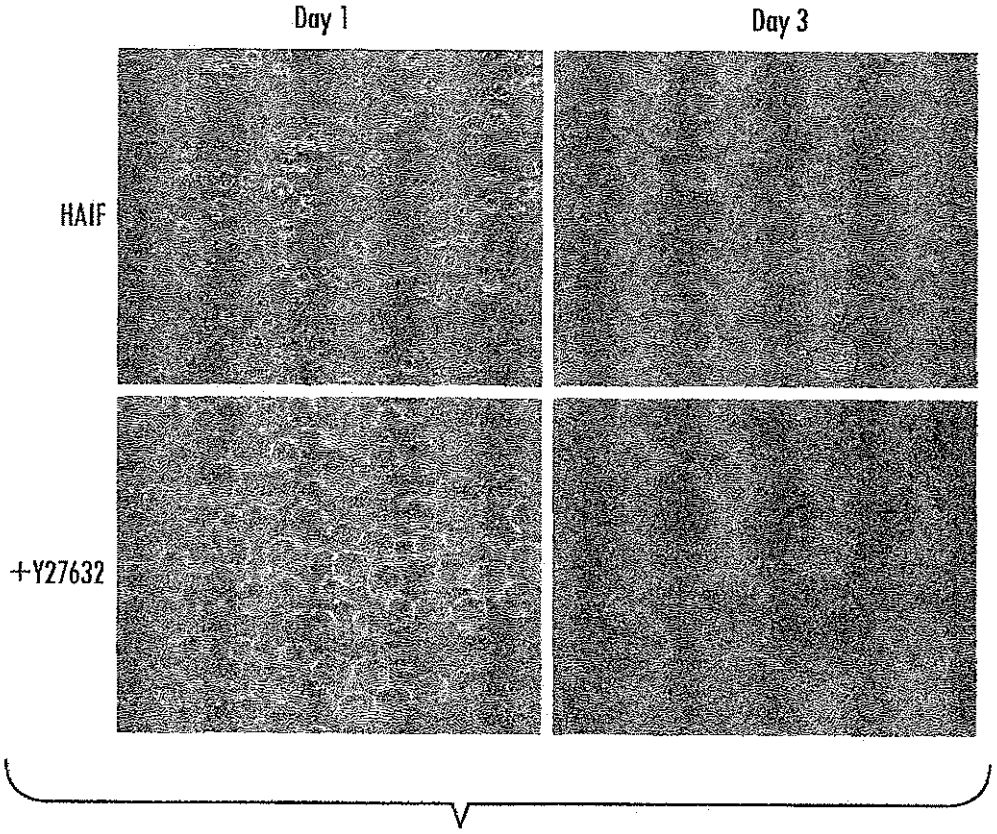


Fig. 20

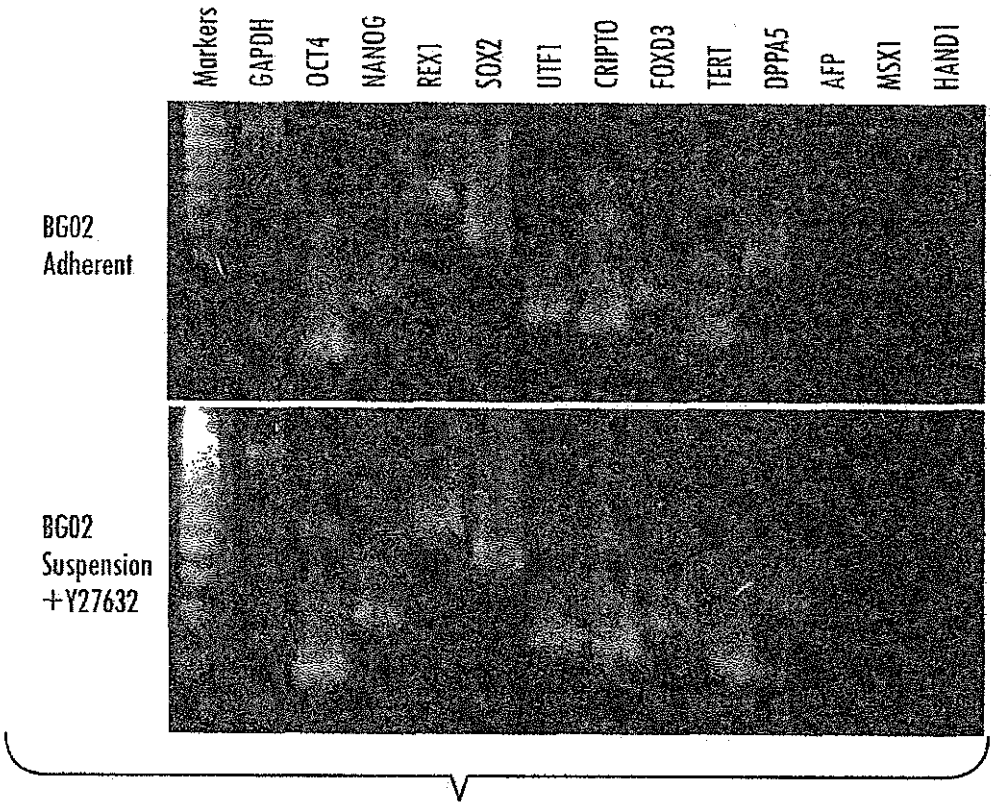

Markers
GAPDH
OCT4
NANOG
REX1
SOX2
UTF1
CRIPTO
FOXD3
TERT
DPPA5
AFP
MSX1
HAND1
BG02
Adherent
BG02
Suspension
+Y27632

Fig. 21

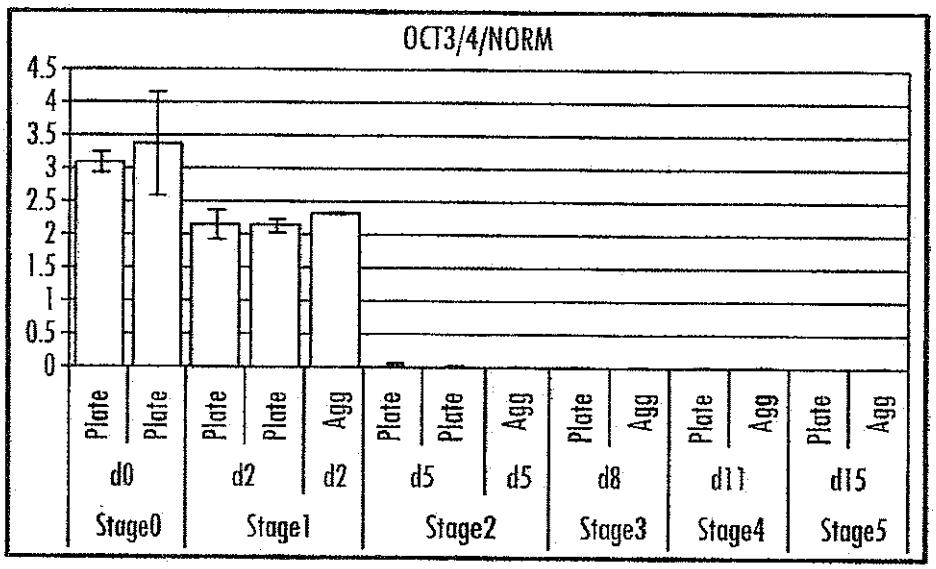


Fig. 22A

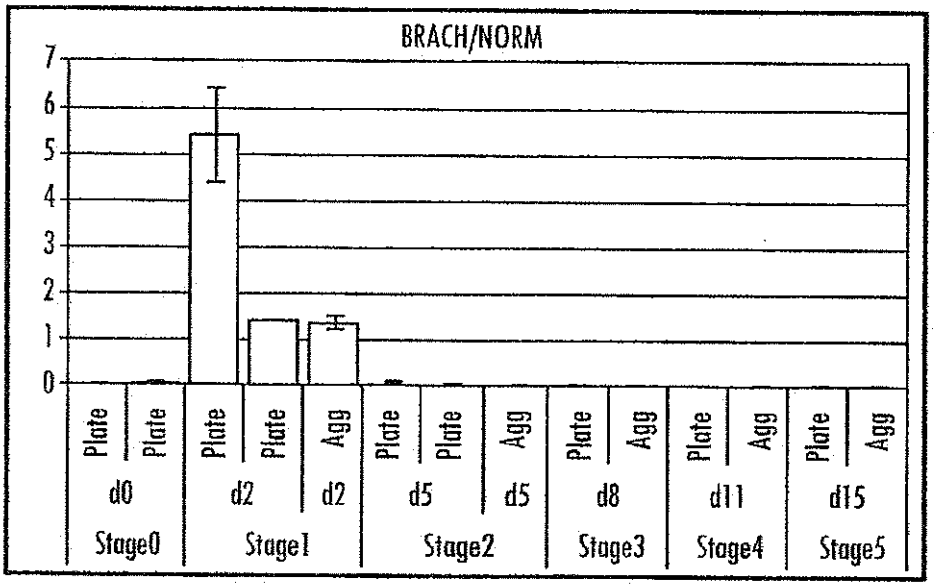


Fig. 22B

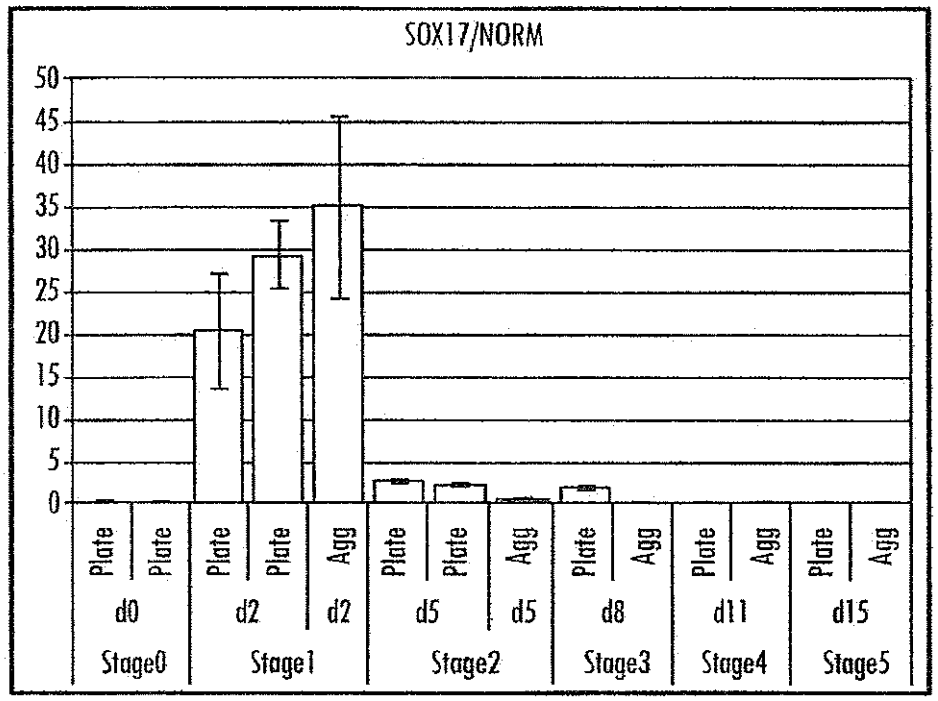


Fig. 22C

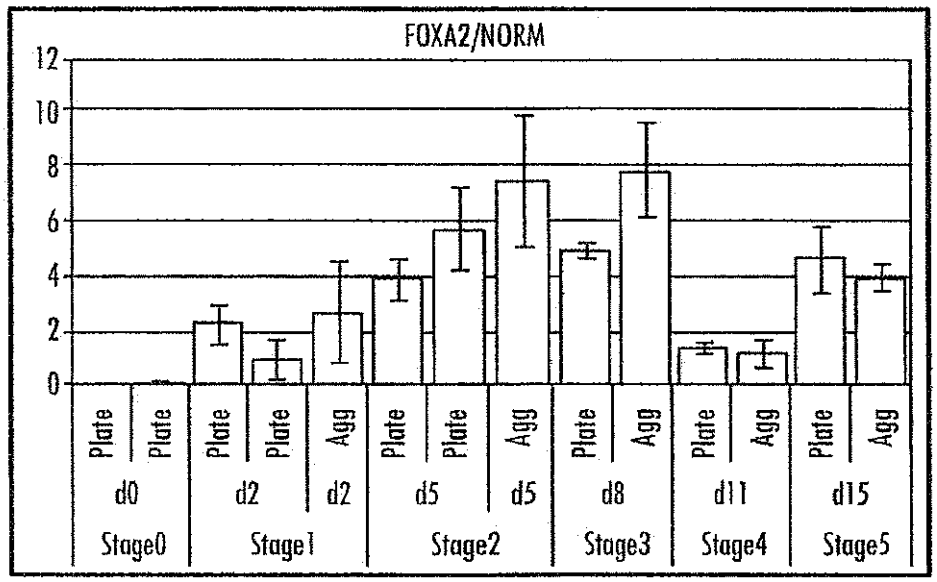


Fig. 22D

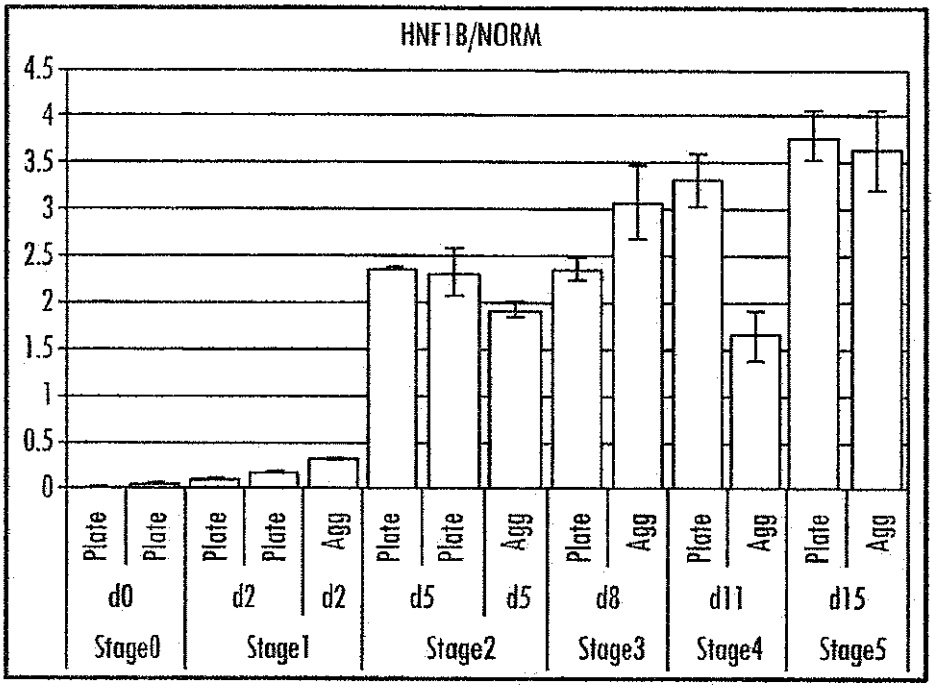


Fig. 22E

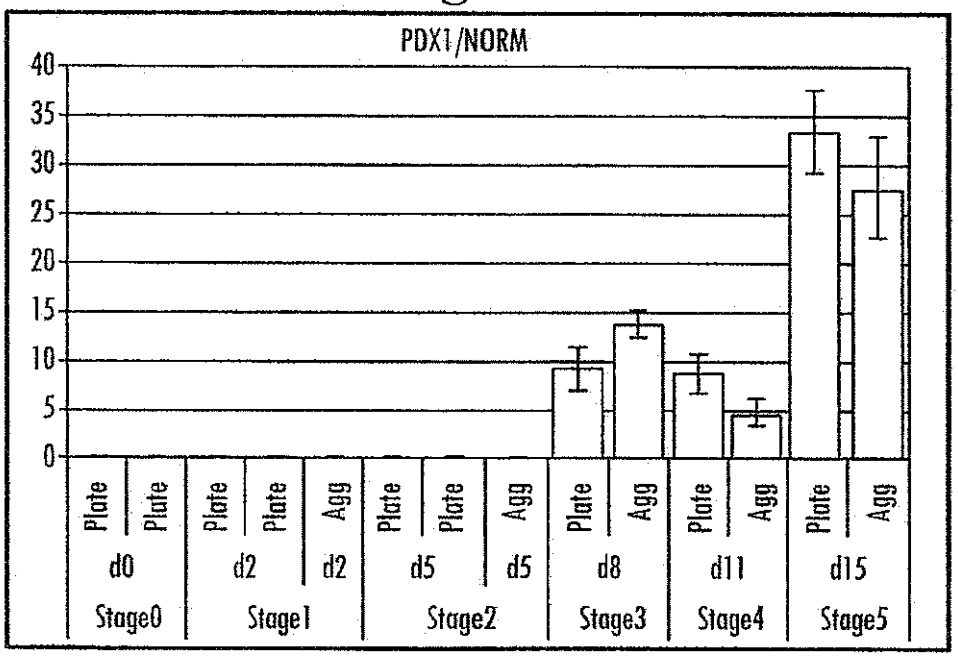


Fig. 22F

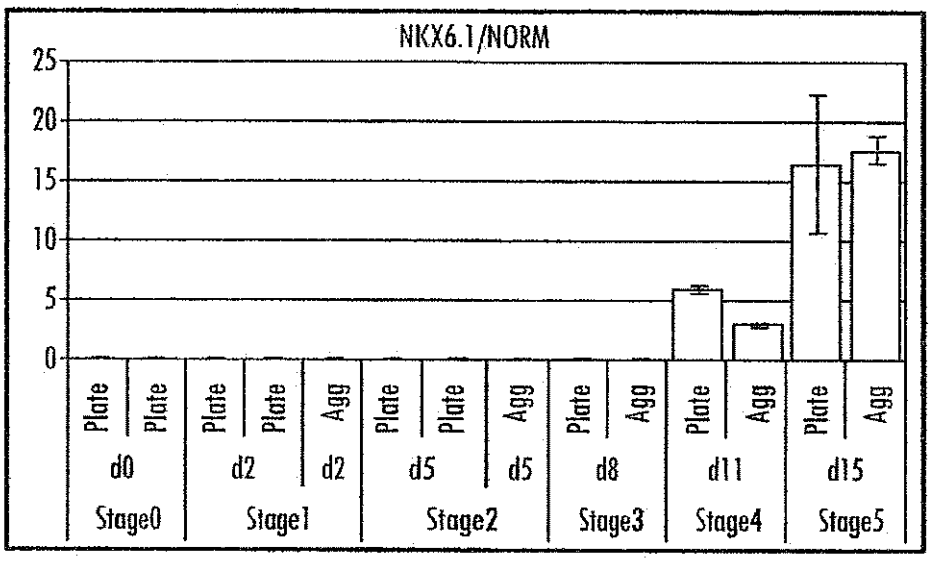


Fig. 22G

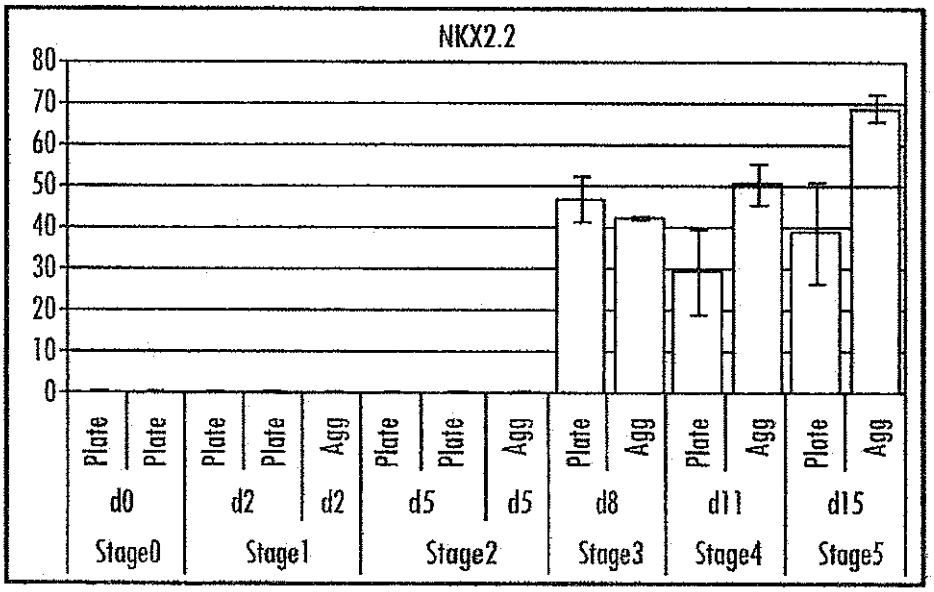


Fig. 22H

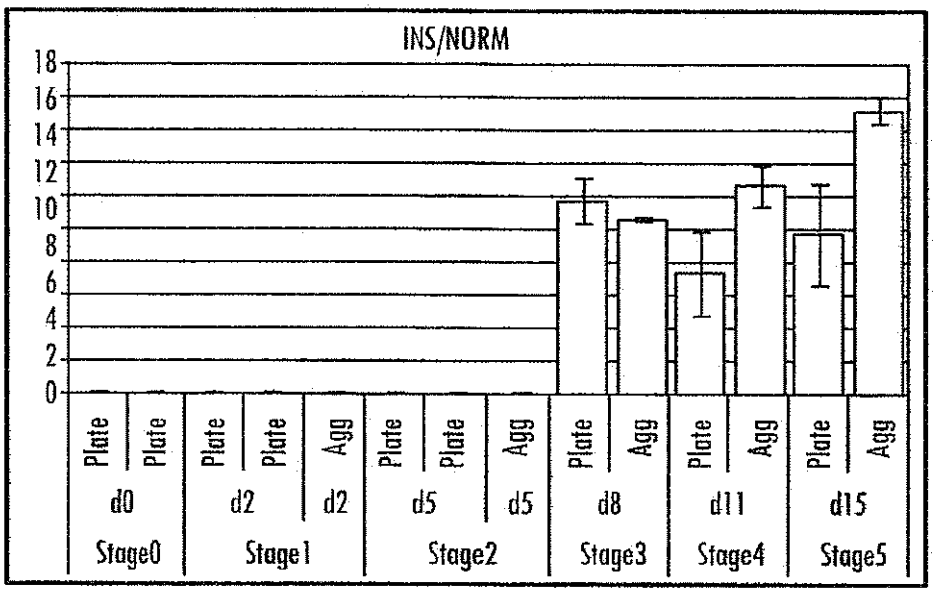


Fig. 22I

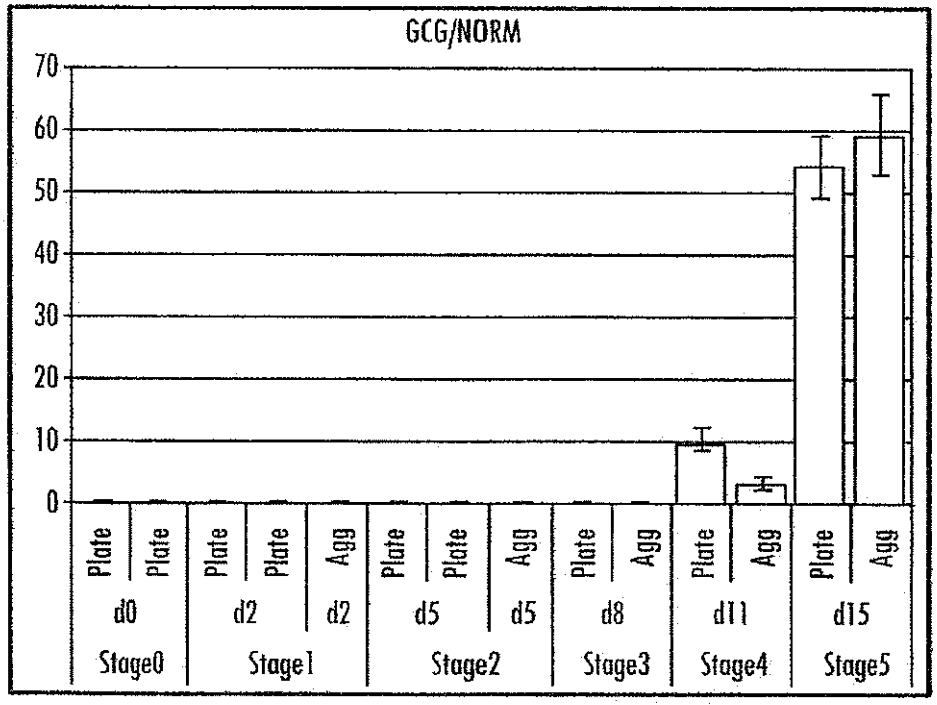


Fig. 22J

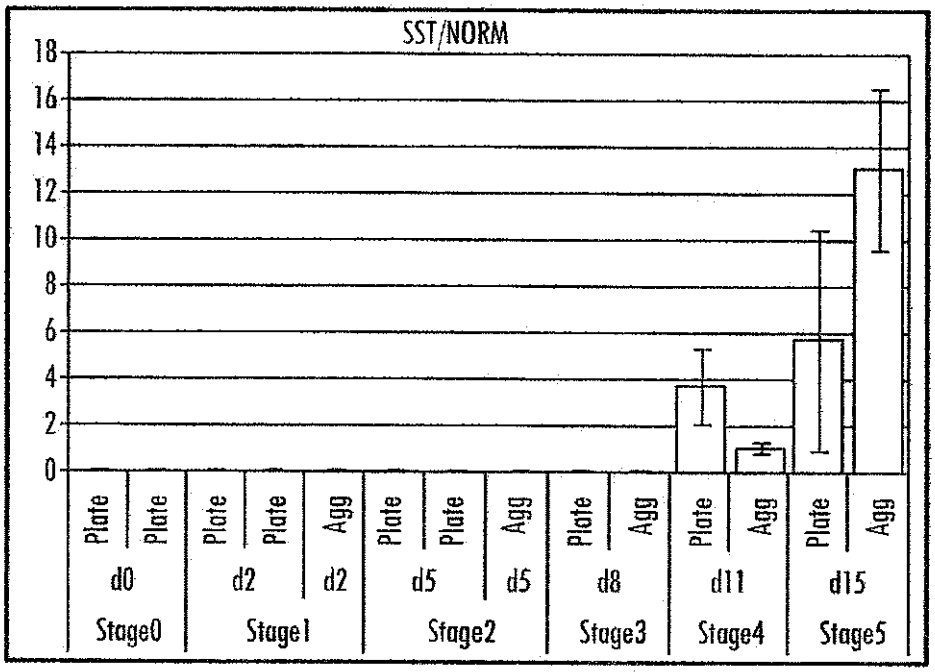


Fig. 22K

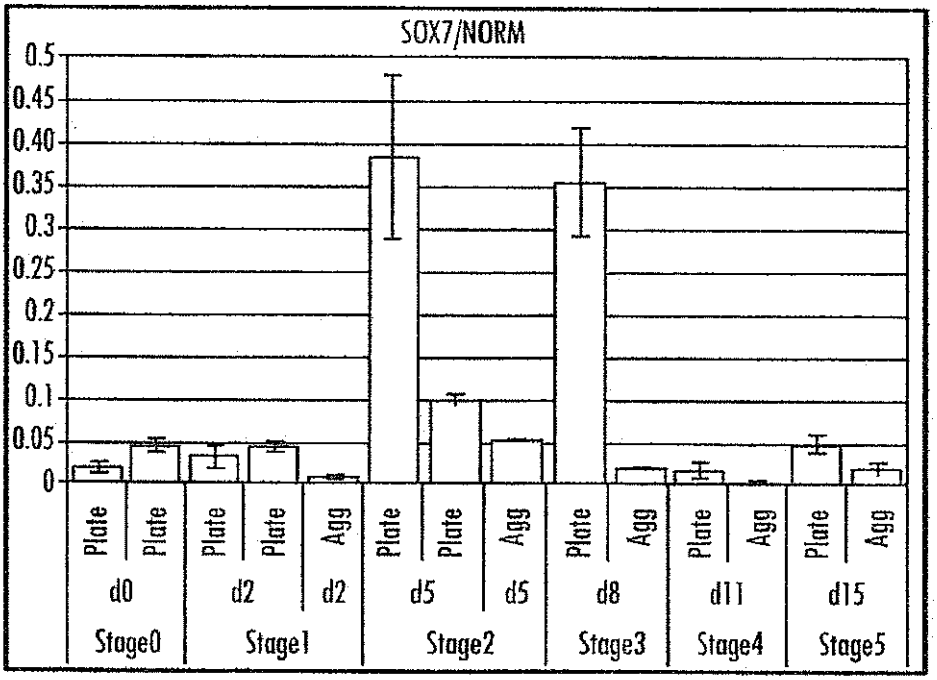


Fig. 22L

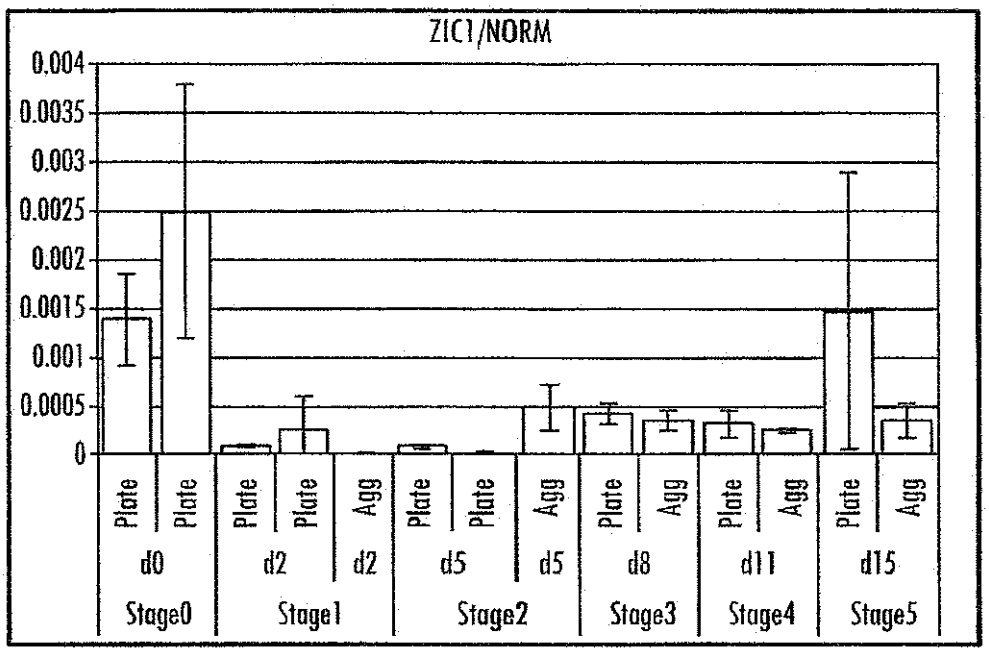


Fig. 22M

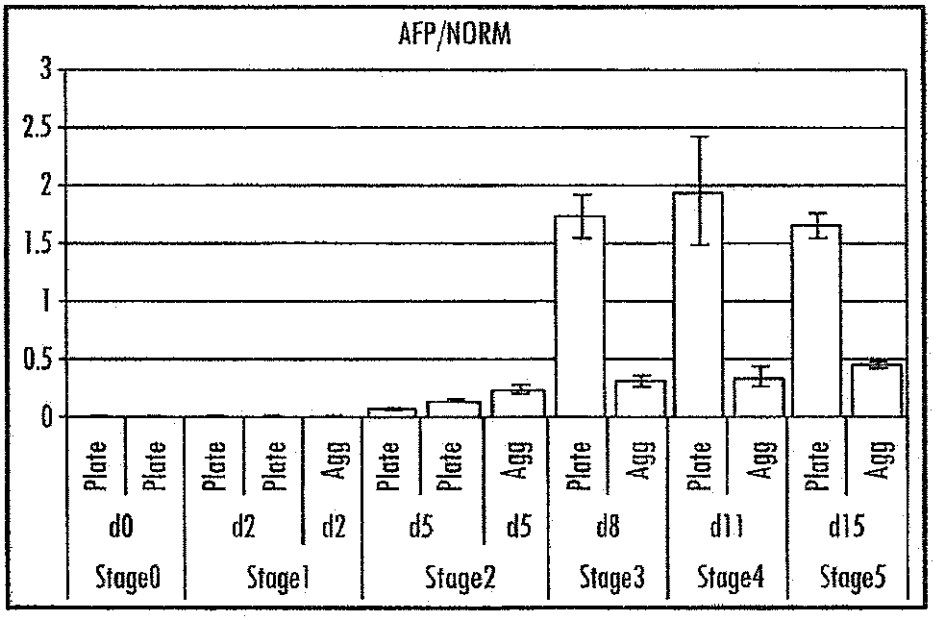


Fig. 22N

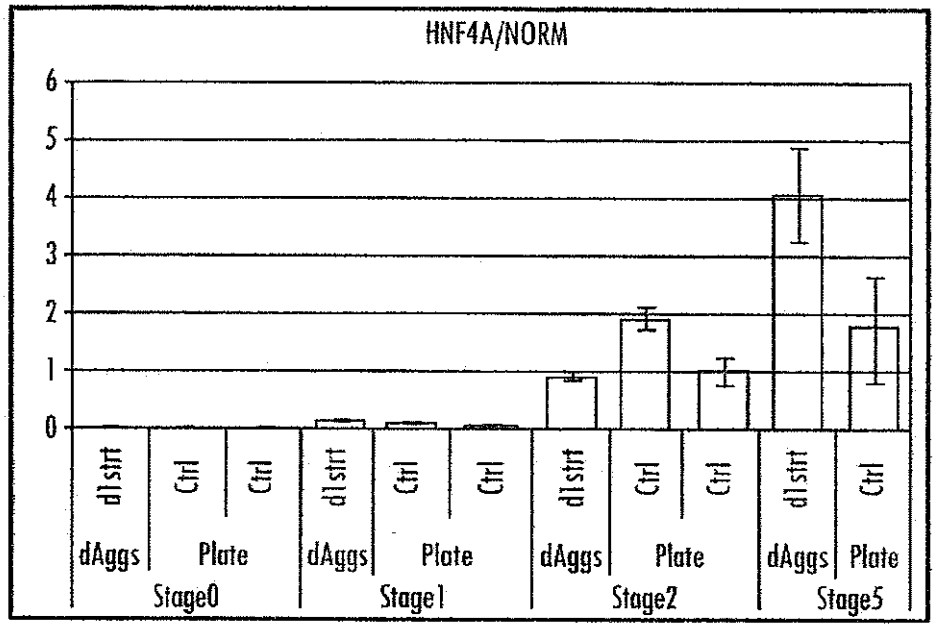


Fig. 22O

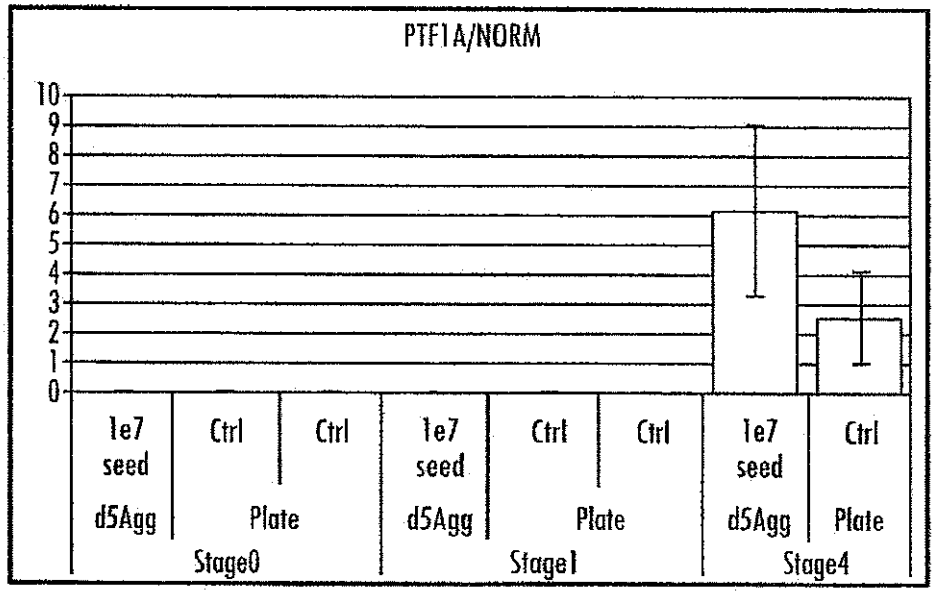


Fig. 22P

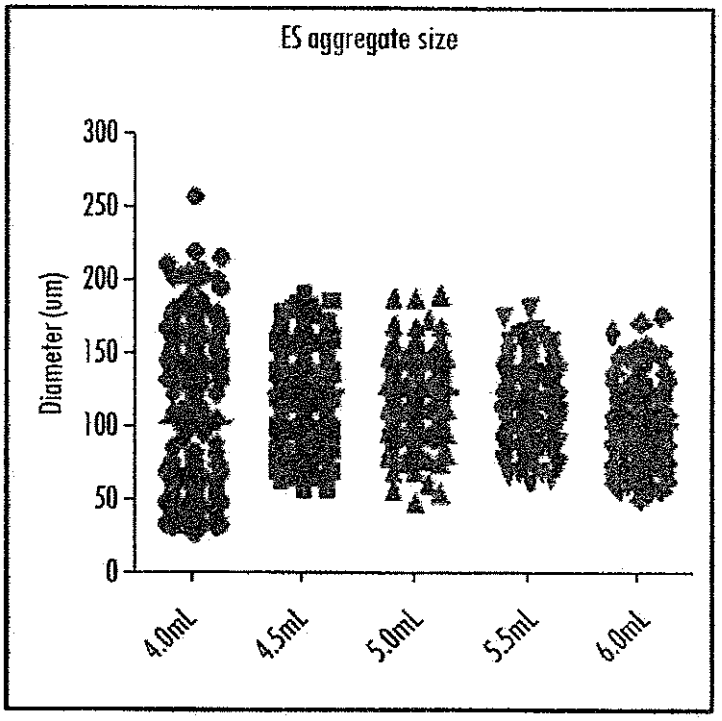

ES aggregate size
Diameter (um)
300
250
200
150
100
50
0
4.0mL
4.5mL
5.0mL
5.5mL
6.0mL

Fig. 23

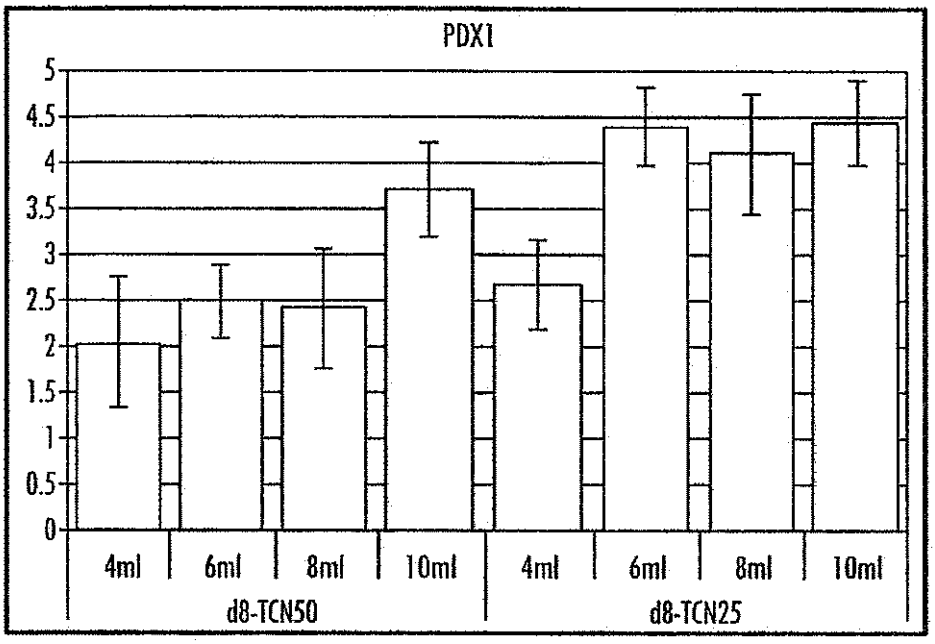


Fig. 24A

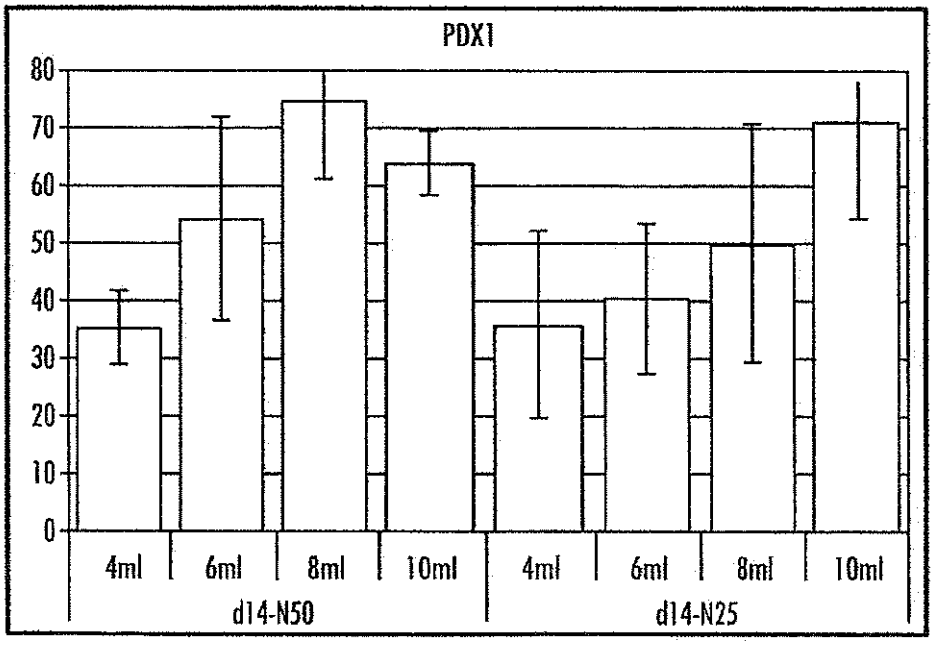


Fig. 24B

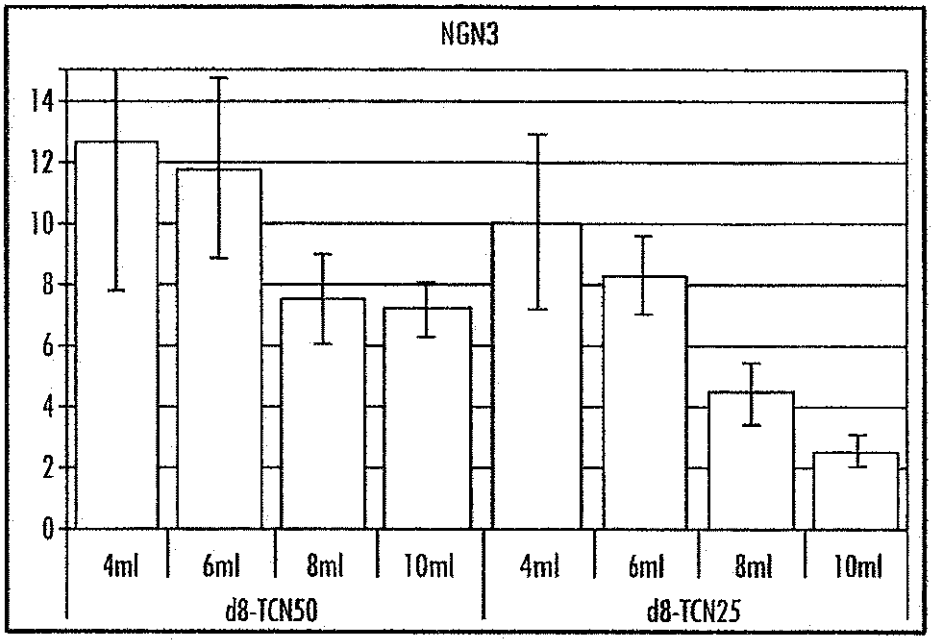


Fig. 24C

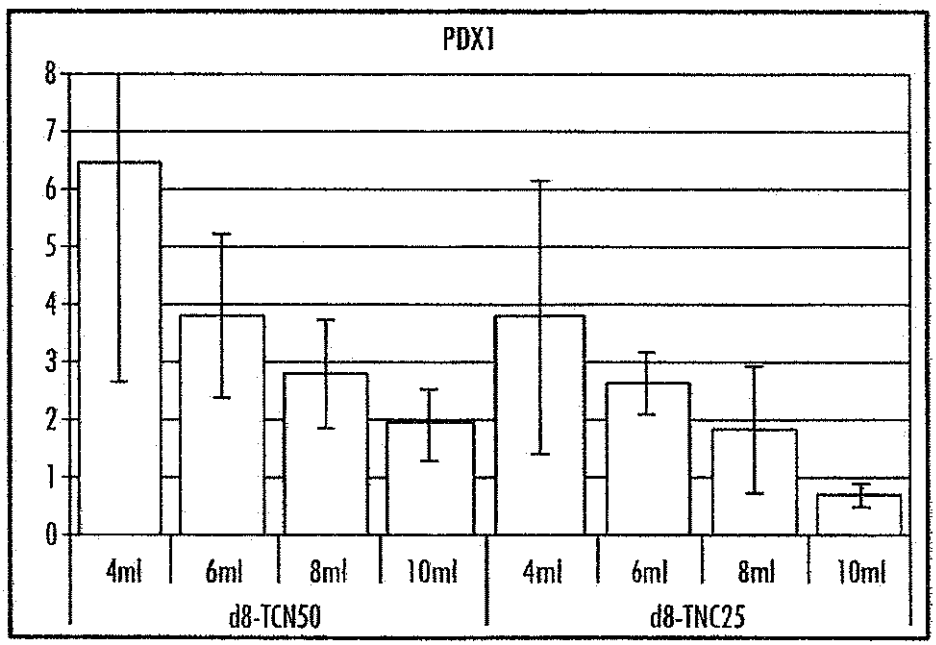


Fig. 24D

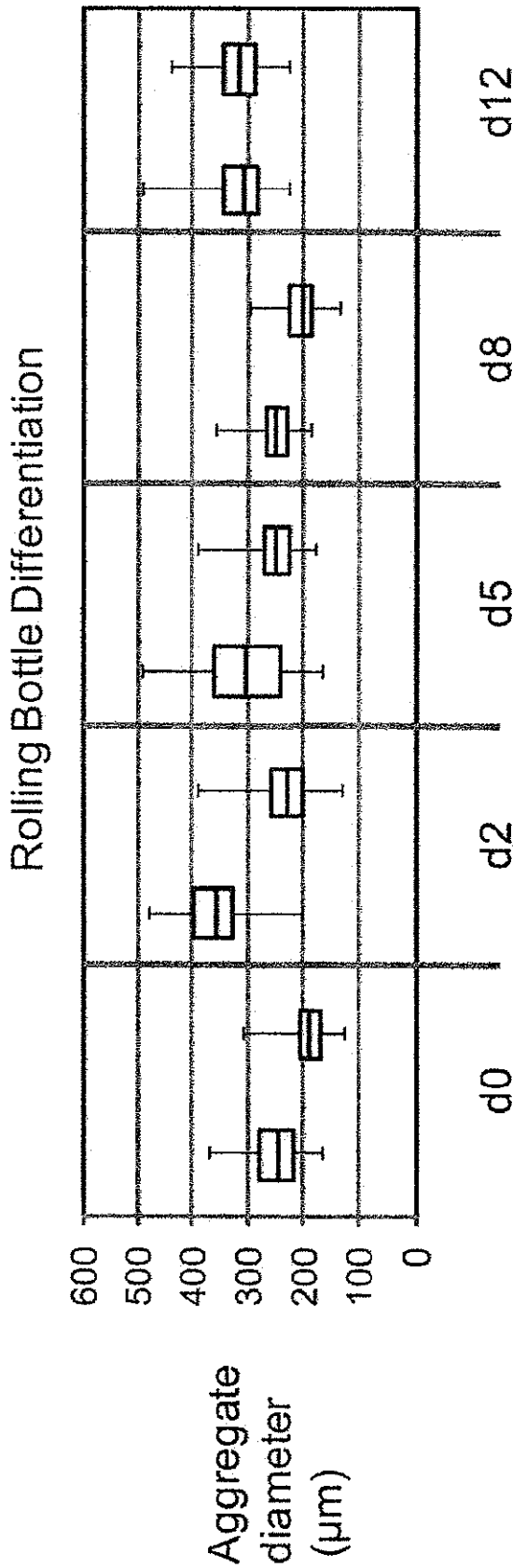

Rolling Bottle Differentiation
Aggregate diameter (µm)
600
500
400
300
200
100
0
d0
d2
d5
d8
d12

FIG. 25

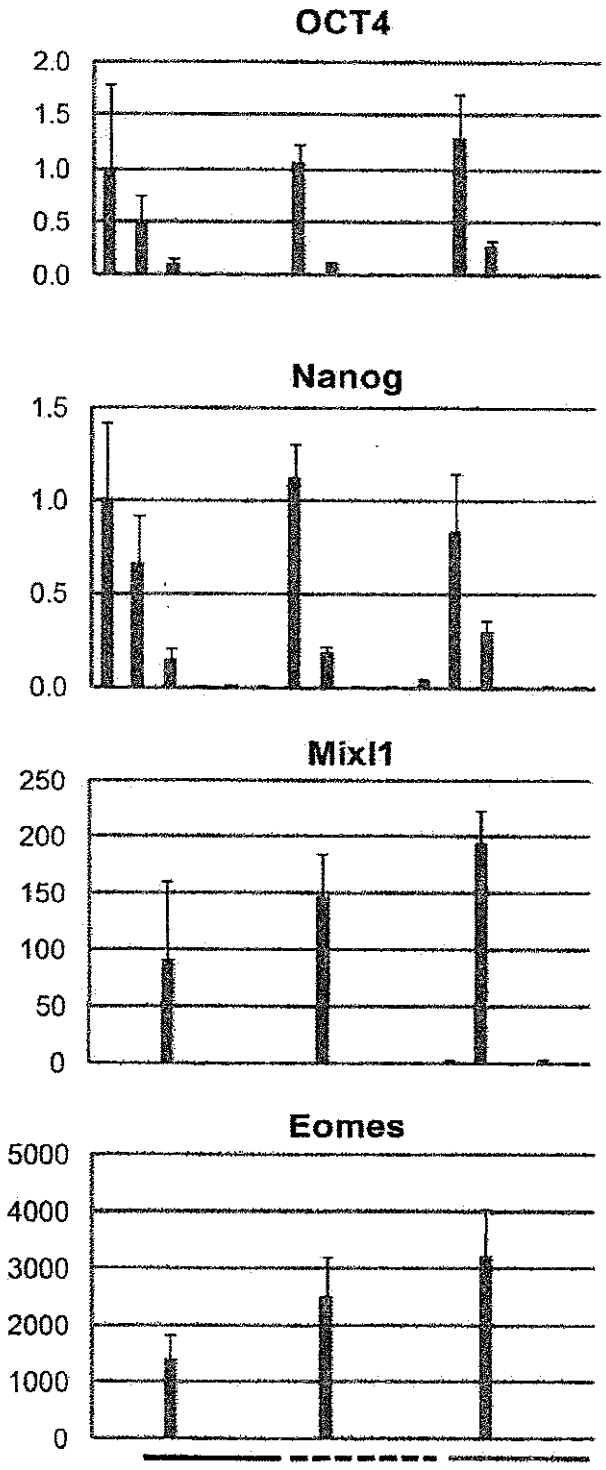

OCT4
2.0
1.5
1.0
0.5
0.0
Nanog
1.5
1.0
0.5
0.0
Mixl1
250
200
150
100
50
0
Eomes
5000
4000
3000
2000
1000
0

FIG. 26A

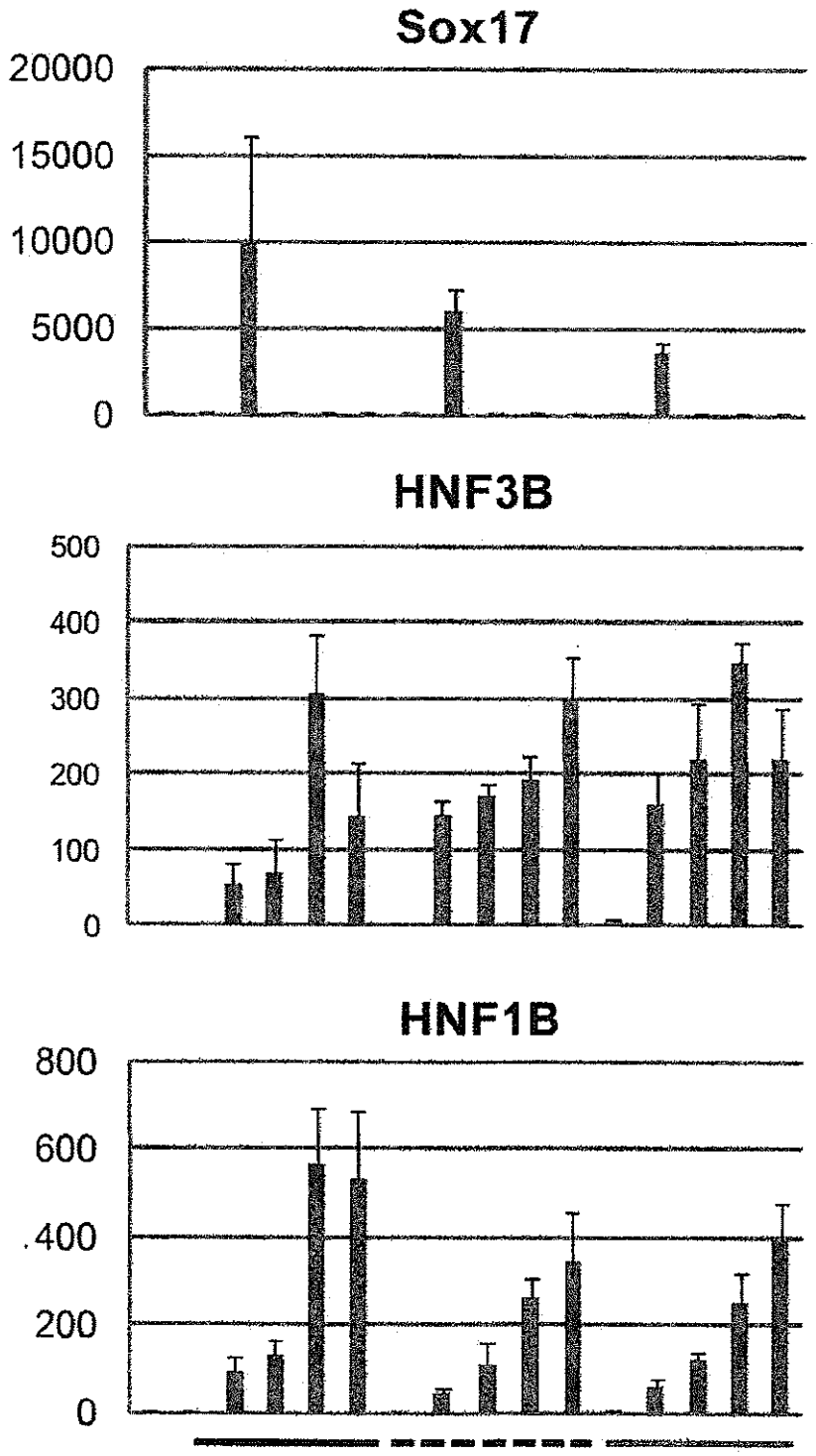

Sox17
20000
15000
10000
5000
0
HNF3B
500
400
300
200
100
0
HNF1B
800
600
400
200
0

FIG. 26B

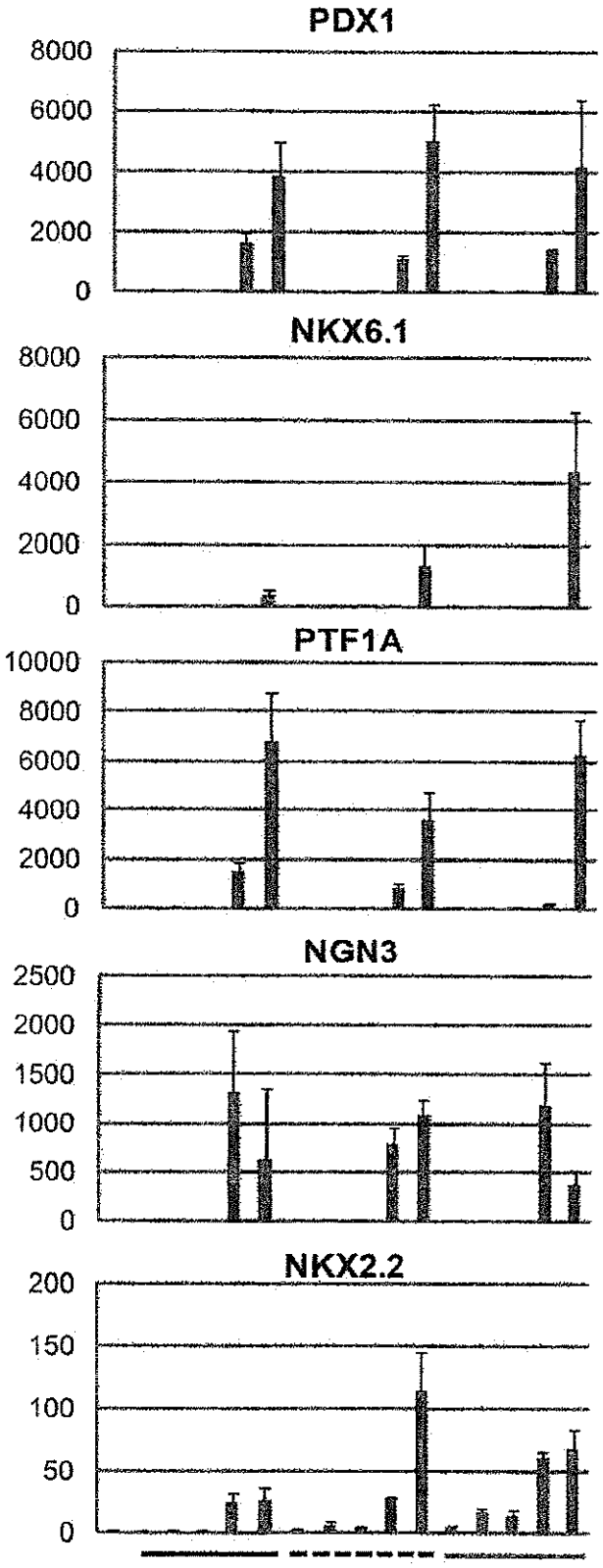

PDX1
8000
6000
4000
2000
0
NKX6.1
8000
6000
4000
2000
0
PTF1A
10000
8000
6000
4000
2000
0
NGN3
2500
2000
1500
1000
500
0
NKX2.2
200
150
100
50
0

FIG. 26C

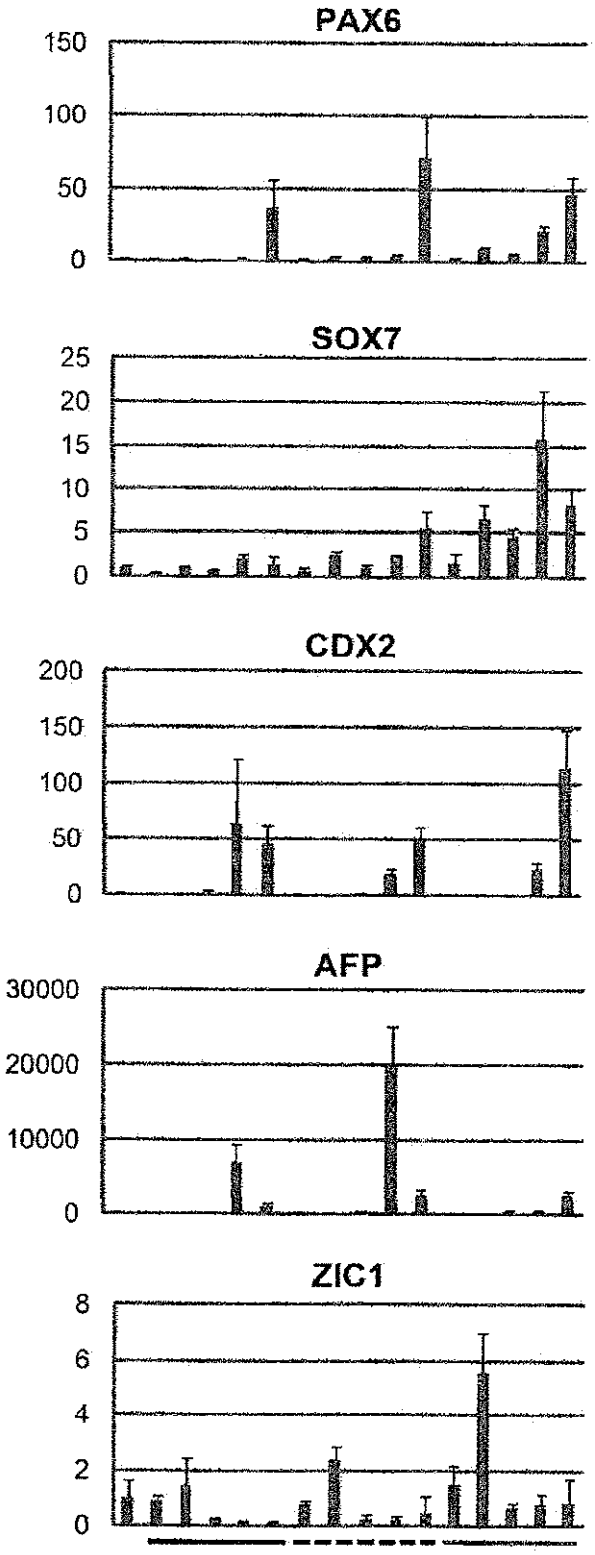

PAX6
150
100
50
0
SOX7
25
20
15
10
5
0
CDX2
200
150
100
50
0
AFP
30000
20000
10000
0
ZIC1
8
6
4
2
0

FIG. 26D

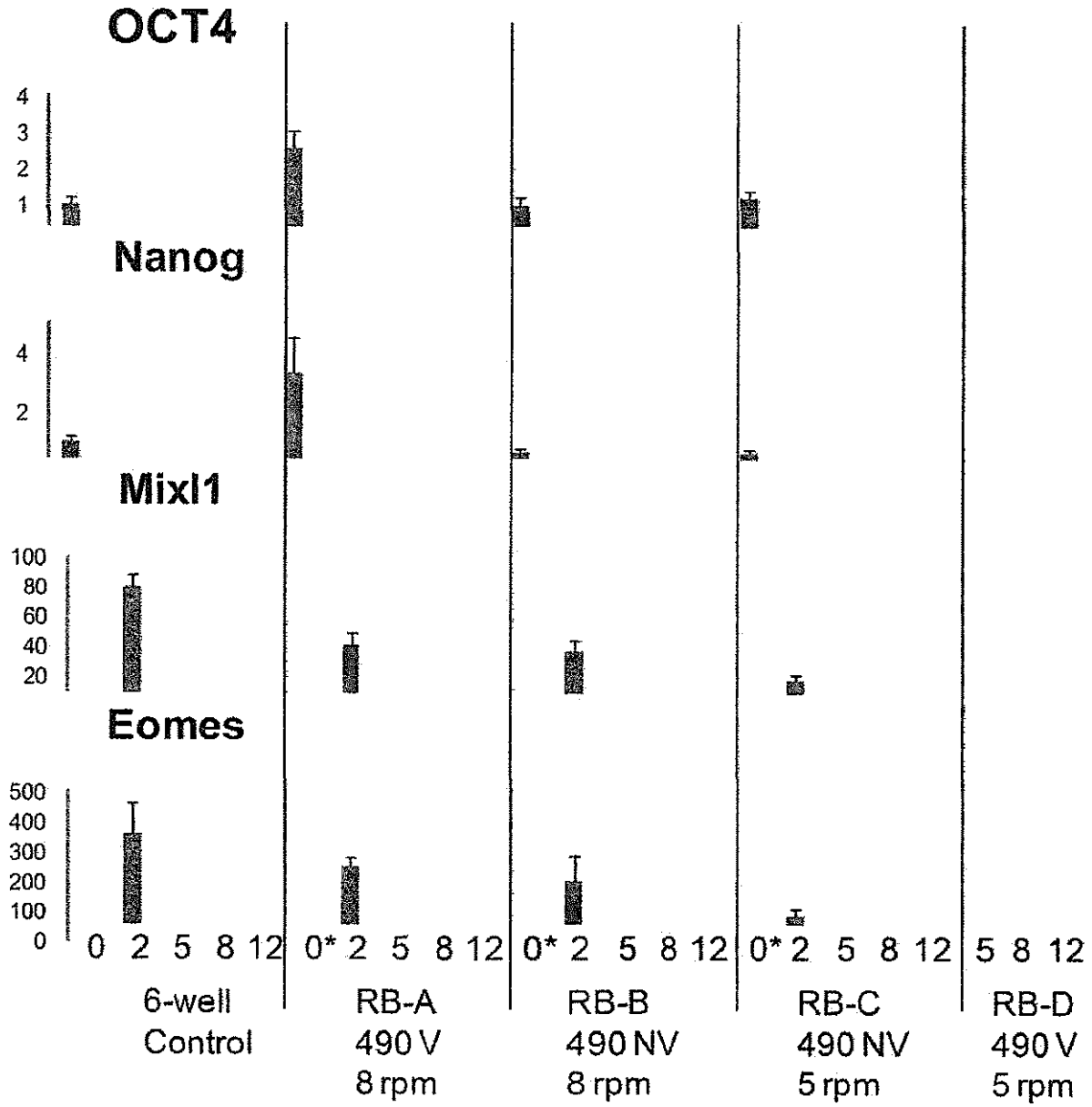

OCT4
4
3
2
1
Nanog
4
2
Mixl1
100
80
60
40
20
Eomes
500
400
300
200
100
0
0 2 5 8 12
0* 2 5 8 12
0* 2 5 8 12
0* 2 5 8 12
5 8 12
6-well Control
RB-A 490 V 8 rpm
RB-B 490 NV 8 rpm
RB-C 490 NV 5 rpm
RB-D 490 V 5 rpm

FIG. 27A

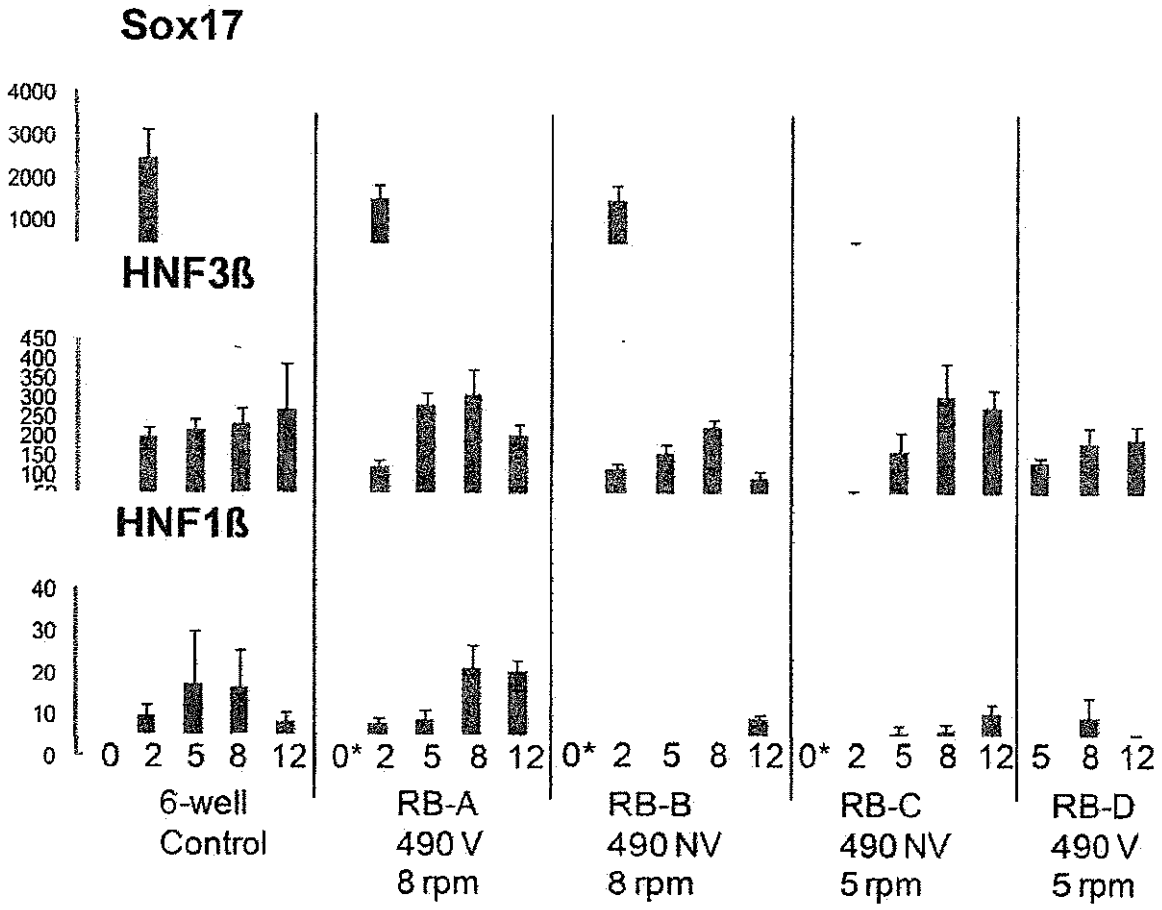

Sox17
4000
3000
2000
1000
HNF3ß
450
400
350
300
250
200
150
100
HNF1ß
40
30
20
10
0
0 2 5 8 12
0* 2 5 8 12
0* 2 5 8 12
0* 2 5 8 12
5 8 12
6-well Control
RB-A 490 V 8 rpm
RB-B 490 NV 8 rpm
RB-C 490 NV 5 rpm
RB-D 490 V 5 rpm

FIG. 27B

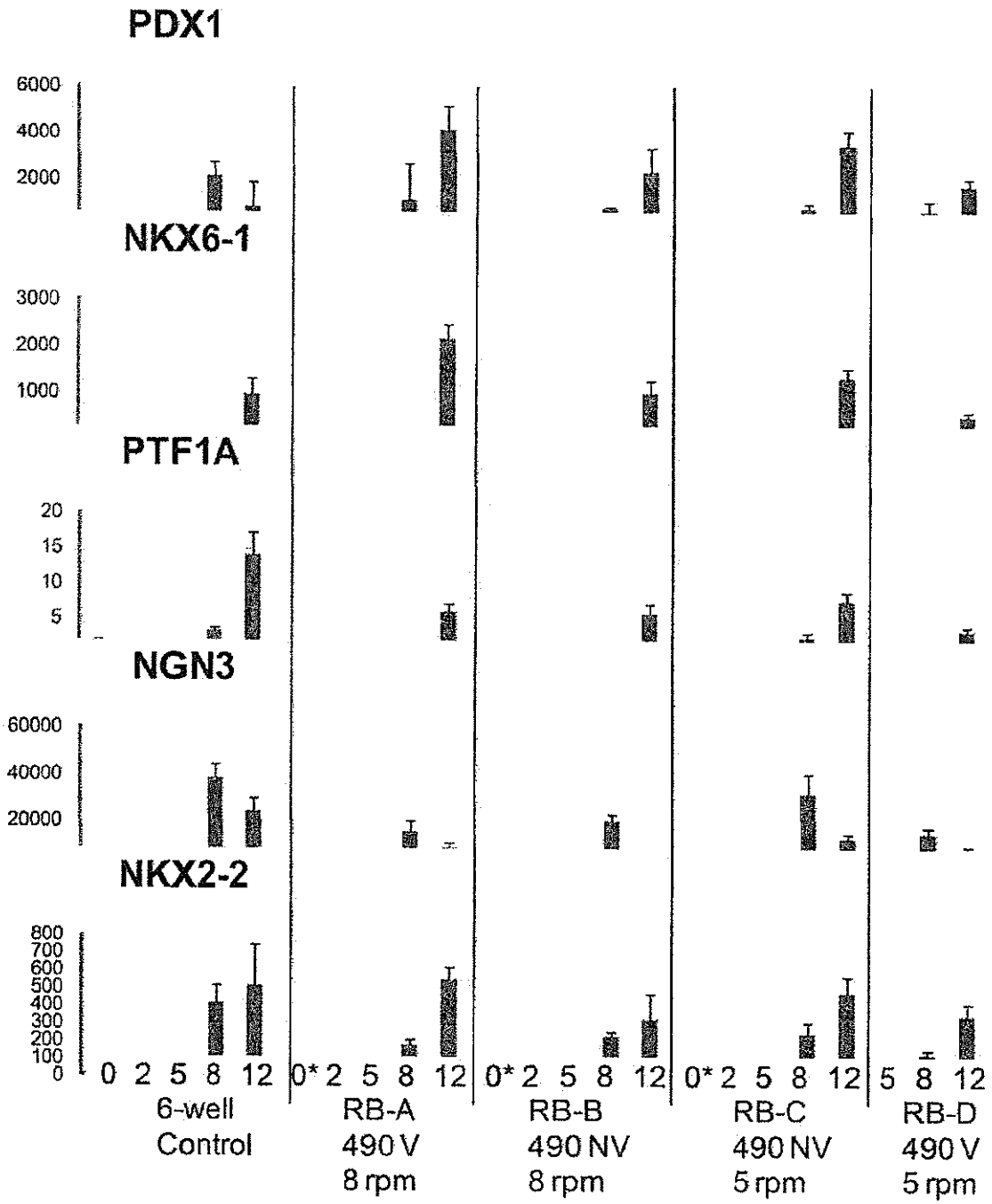

PDX1
6000
4000
2000
NKX6-1
3000
2000
1000
PTF1A
20
15
10
5
NGN3
60000
40000
20000
NKX2-2
800
700
600
500
400
300
200
100
0
0 2 5 8 12
0* 2 5 8 12
0* 2 5 8 12
0* 2 5 8 12
5 8 12
6-well Control
RB-A 490 V 8 rpm
RB-B 490 NV 8 rpm
RB-C 490 NV 5 rpm
RB-D 490 V 5 rpm

FIG. 27C

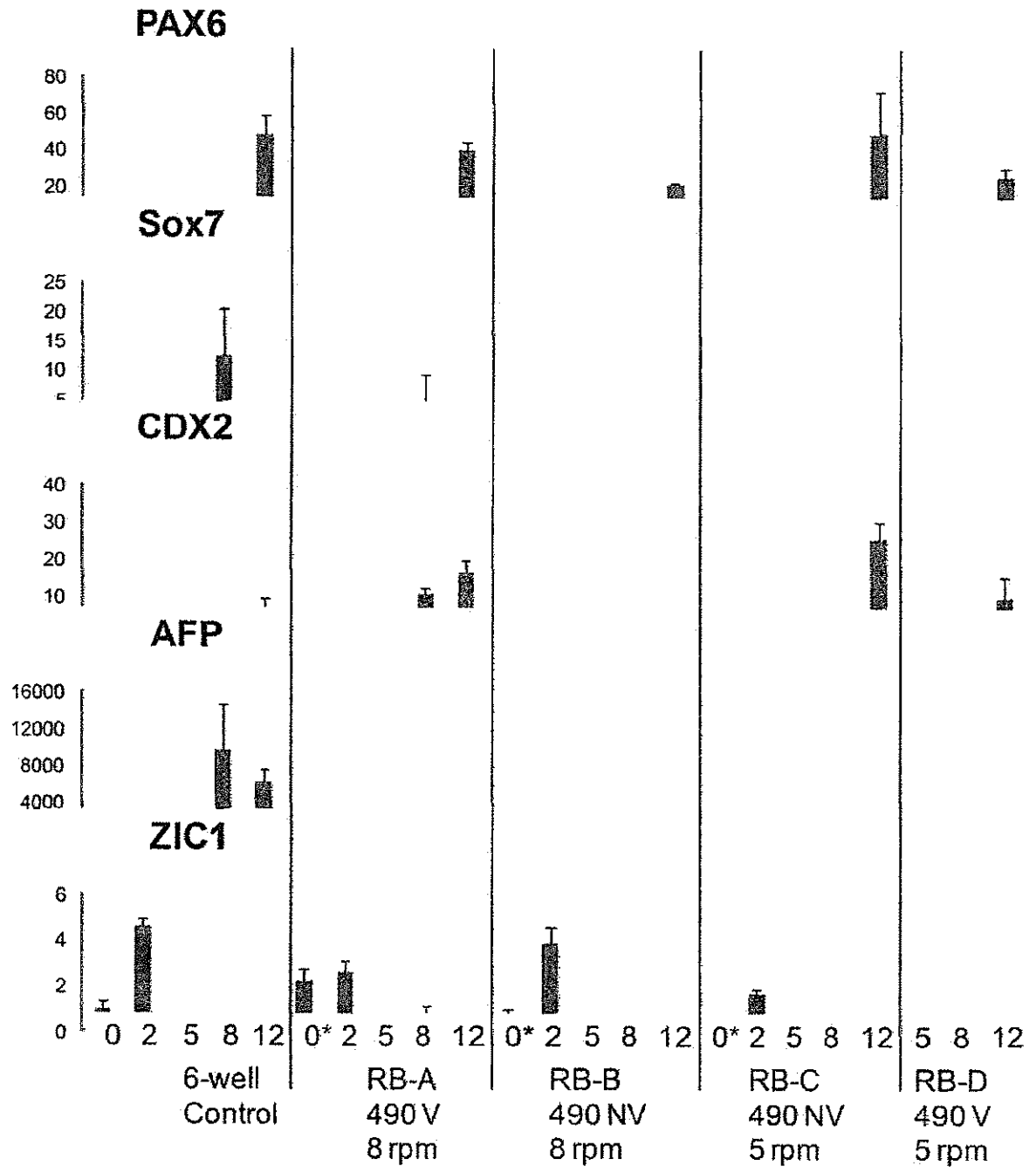

PAX6
80
60
40
20
Sox7
25
20
15
10
CDX2
40
30
20
10
AFP
16000
12000
8000
4000
ZIC1
6
4
2
0
0 2 5 8 12
0* 2 5 8 12
0* 2 5 8 12
0* 2 5 8 12
5 8 12
6-well Control
RB-A 490 V 8 rpm
RB-B 490 NV 8 rpm
RB-C 490 NV 5 rpm
RB-D 490 V 5 rpm

FIG. 27D