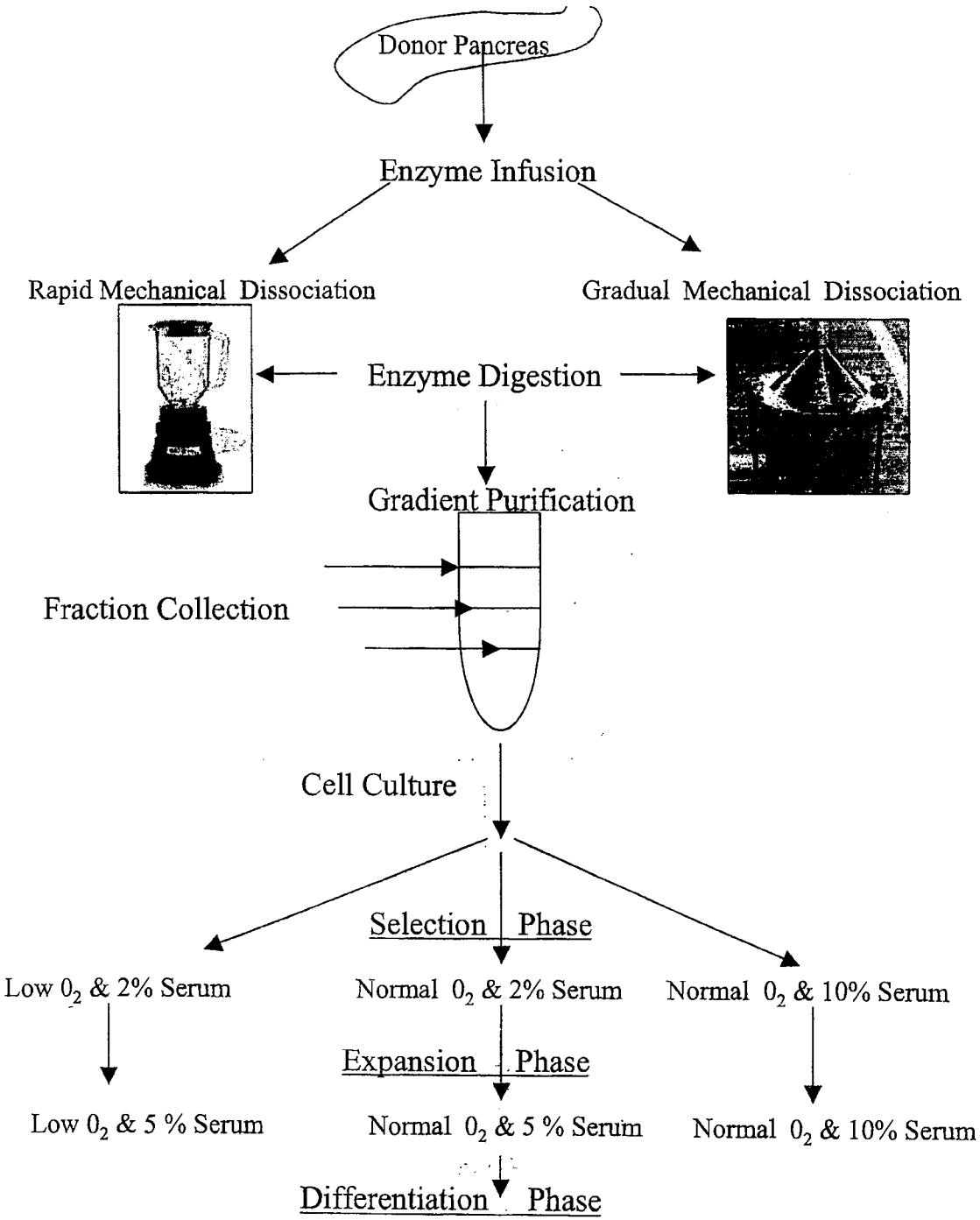
Figure 1


Donor Pancreas
Enzyme Infusion
Rapid Mechanical Dissociation
Gradual Mechanical Dissociation
Enzyme Digestion
Gradient Purification
Fraction Collection
Cell Culture
Selection Phase
Low 0_2 & 2% Serum
Normal 0_2 & 2% Serum
Normal 0_2 & 10% Serum
Expansion Phase
Low 0_2 & 5 % Serum
Normal 0_2 & 5 % Serum
Normal 0_2 & 10% Serum
Differentiation Phase

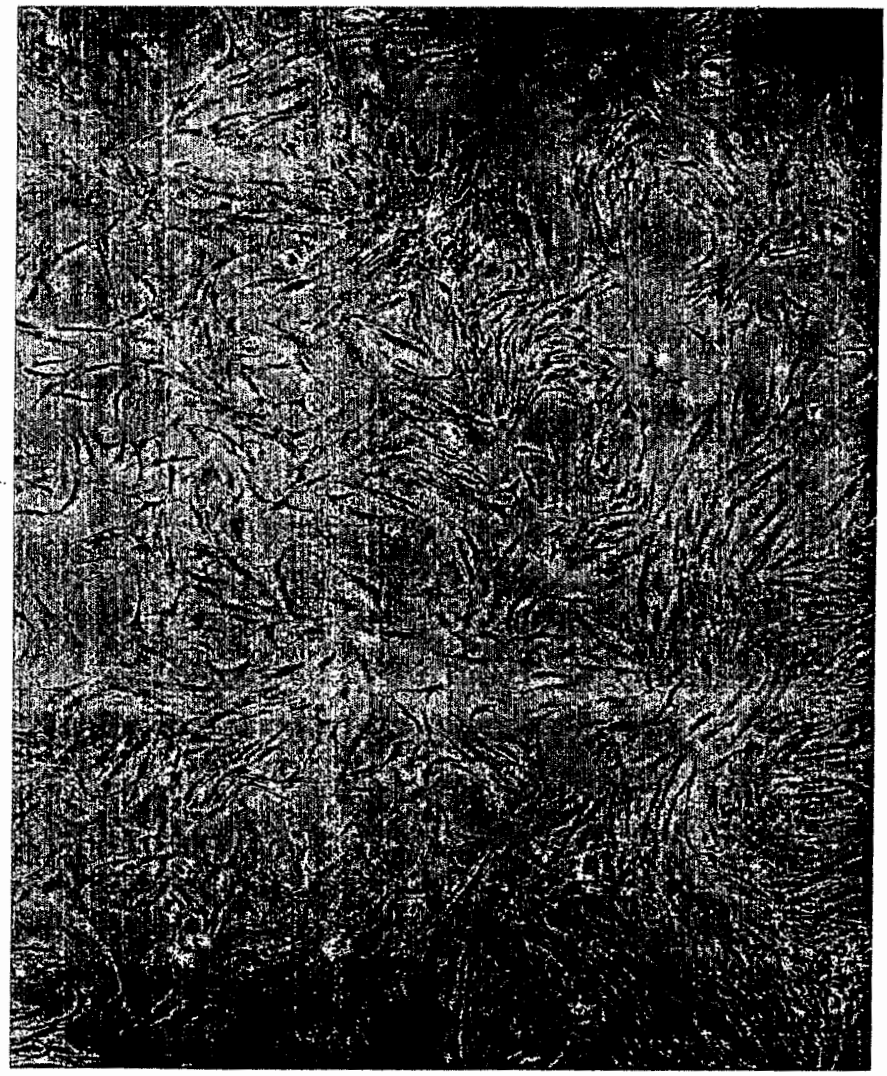


Figure 2

Figure 3

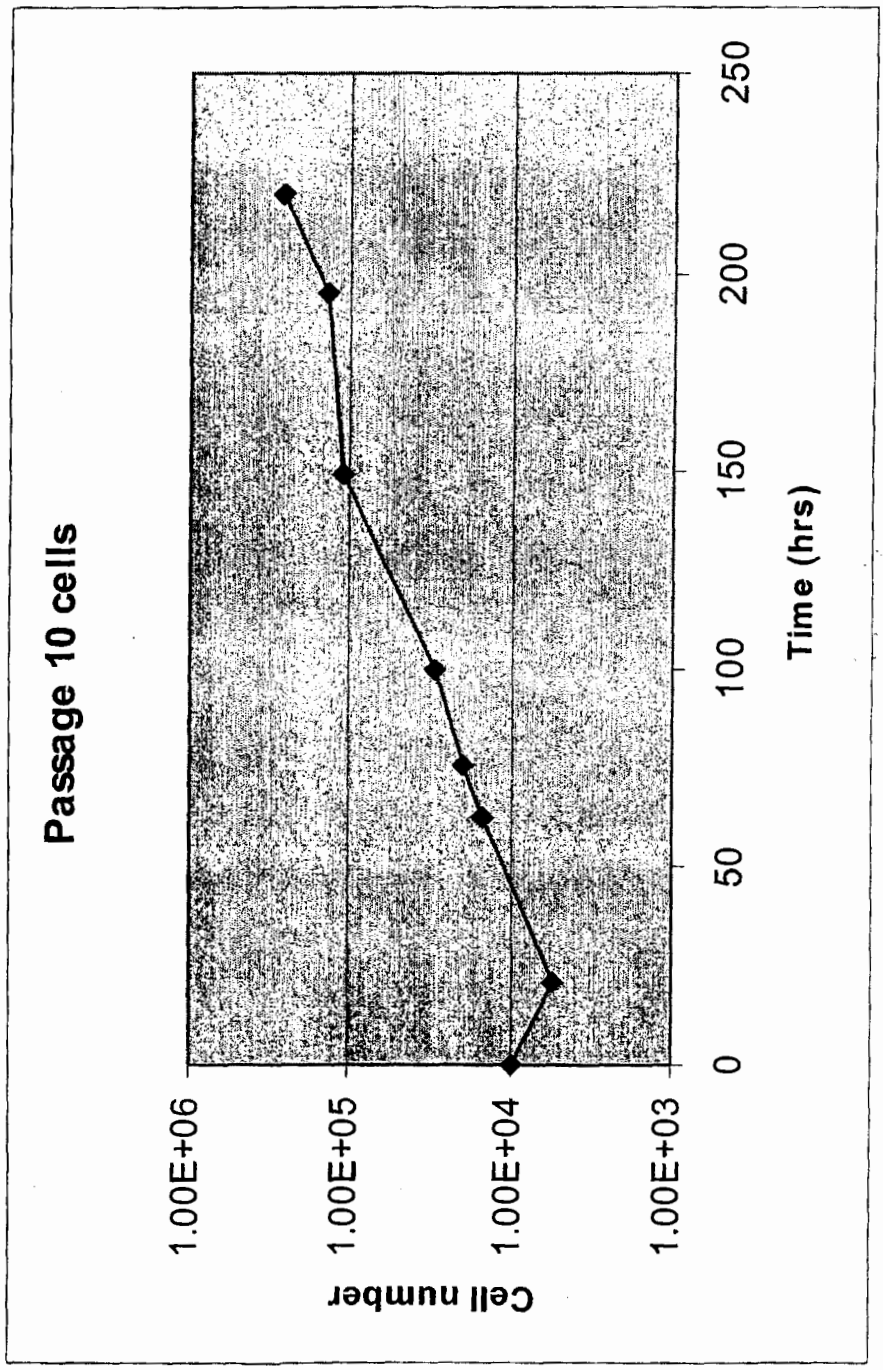

Passage 10 cells
Cell number
1.00E+06
1.00E+05
1.00E+04
1.00E+03
0
50
100
150
200
250
Time (hrs)

Figure 4

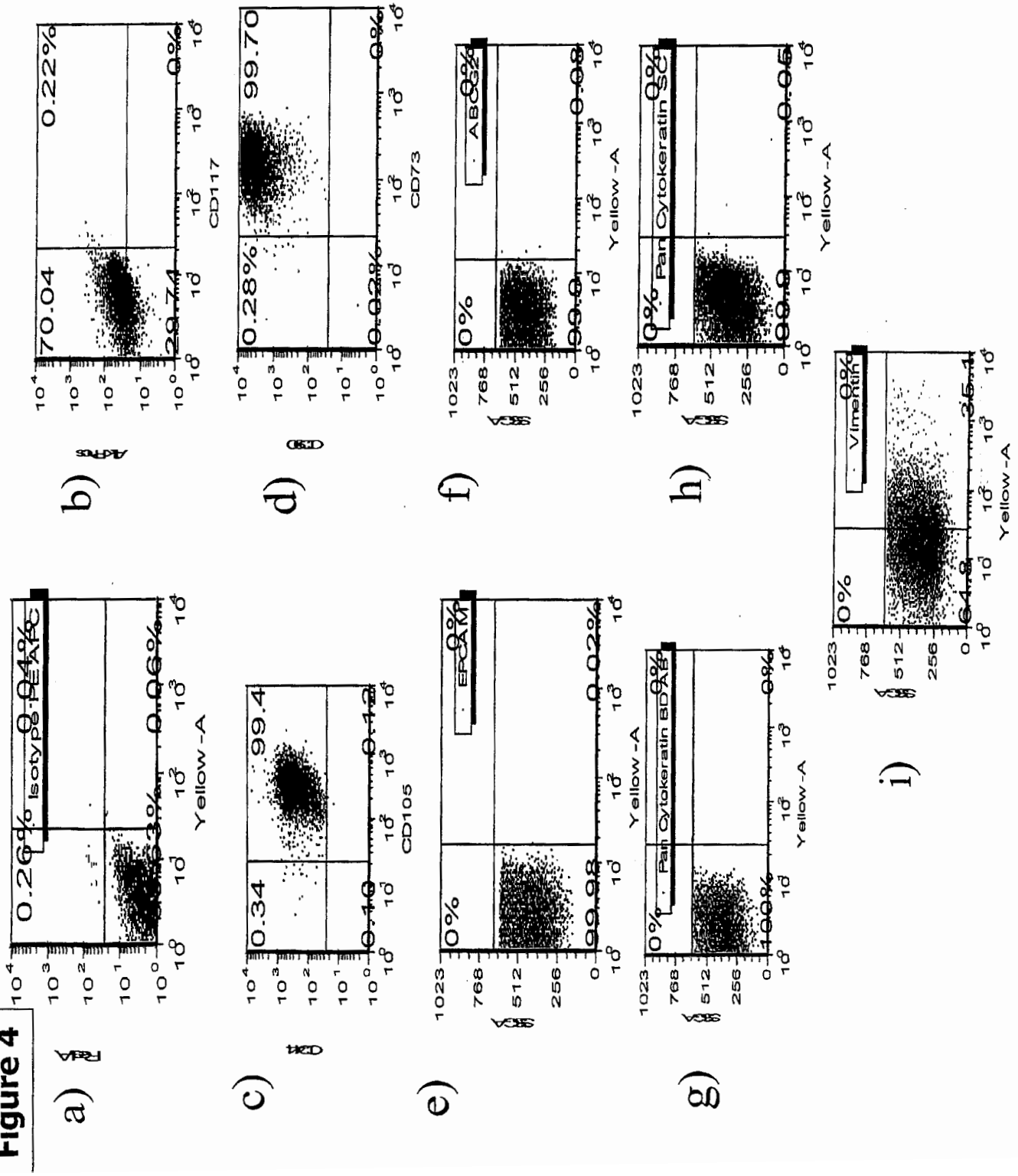

a)
b)
c)
d)
e)
f)
g)
h)
i)
Yellow-A
CD117
CD73
CD105
Isotype PE/APC
EPCAM
ABCG2
Pan Cytokeratin BD
Pan Cytokeratin SC
Vimentin
0.26%
0.04%
0.06%
70.04
0.22%
29.74
0.28%
99.70
0.02%
0.34
99.4
0.12
0%
99.98
100%
35.1

Figure 5

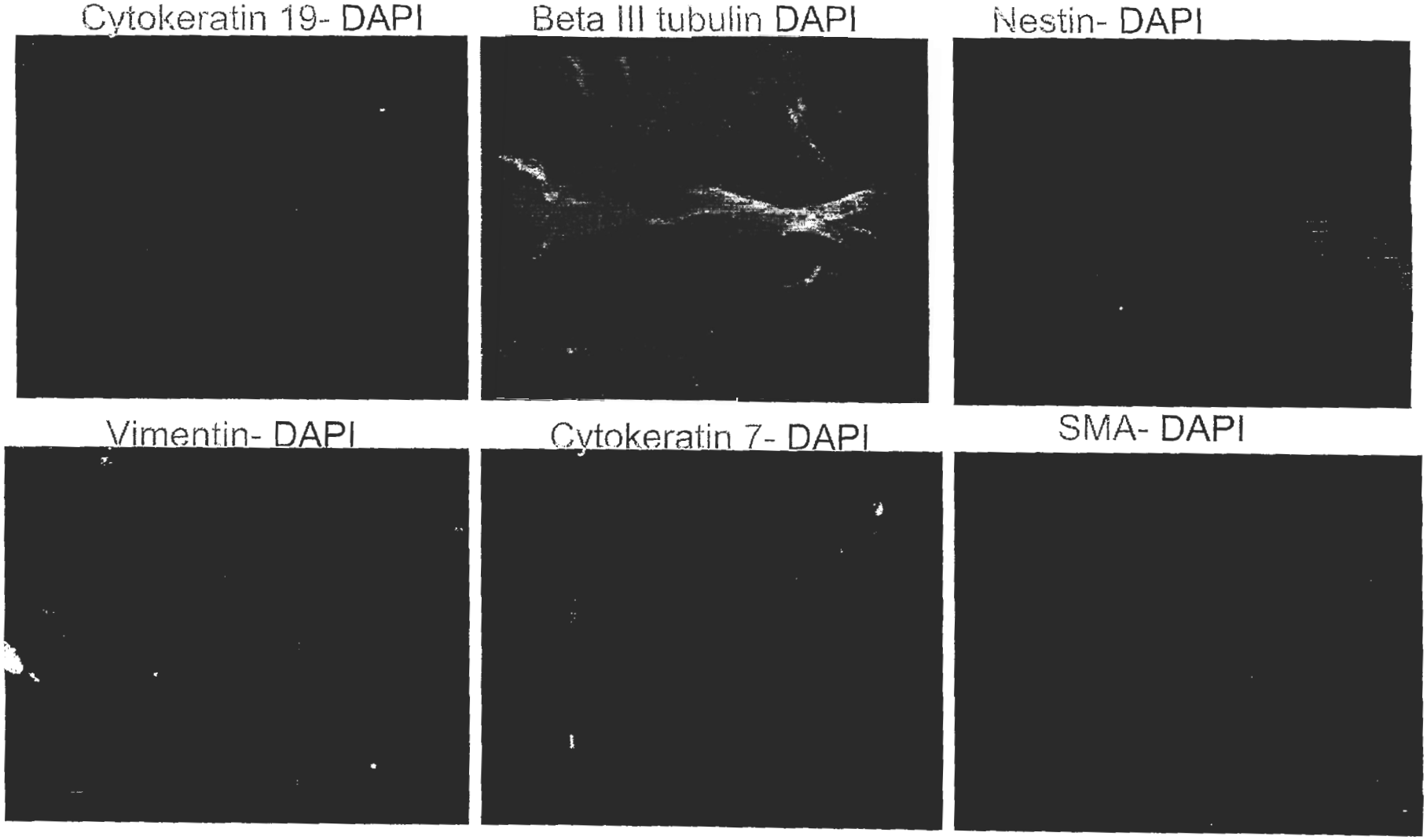

Cytokeratin 19- DAPI
Beta III tubulin DAPI
Nestin- DAPI
Vimentin- DAPI
Cytokeratin 7- DAPI
SMA- DAPI

Figure 6

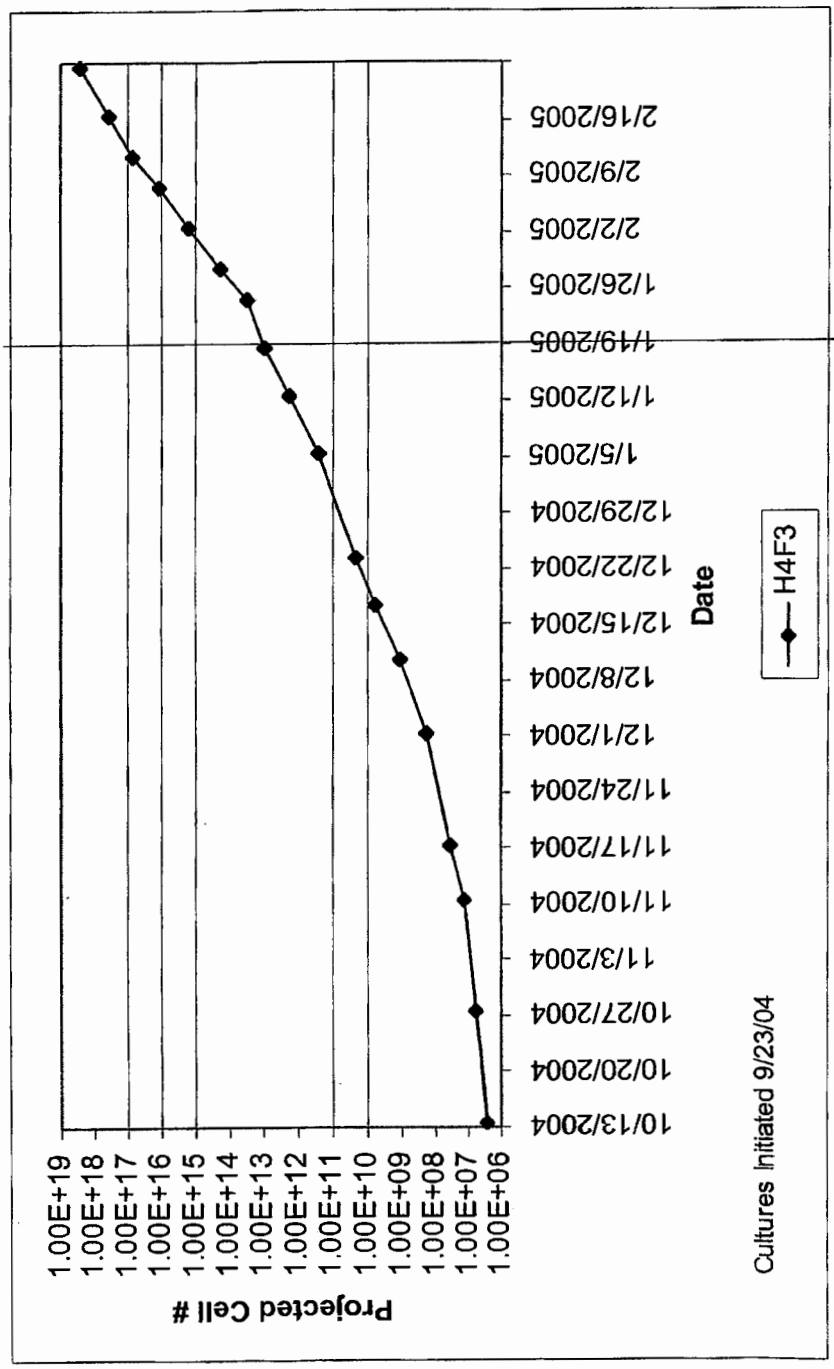

Projected Cell #
1.00E+19
1.00E+18
1.00E+17
1.00E+16
1.00E+15
1.00E+14
1.00E+13
1.00E+12
1.00E+11
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
10/13/2004
10/20/2004
10/27/2004
11/3/2004
11/10/2004
11/17/2004
11/24/2004
12/1/2004
12/8/2004
12/15/2004
12/22/2004
12/29/2004
1/5/2005
1/12/2005
1/19/2005
1/26/2005
2/2/2005
2/9/2005
2/16/2005
Date
H4F3
Cultures Initiated 9/23/04

Figure 7

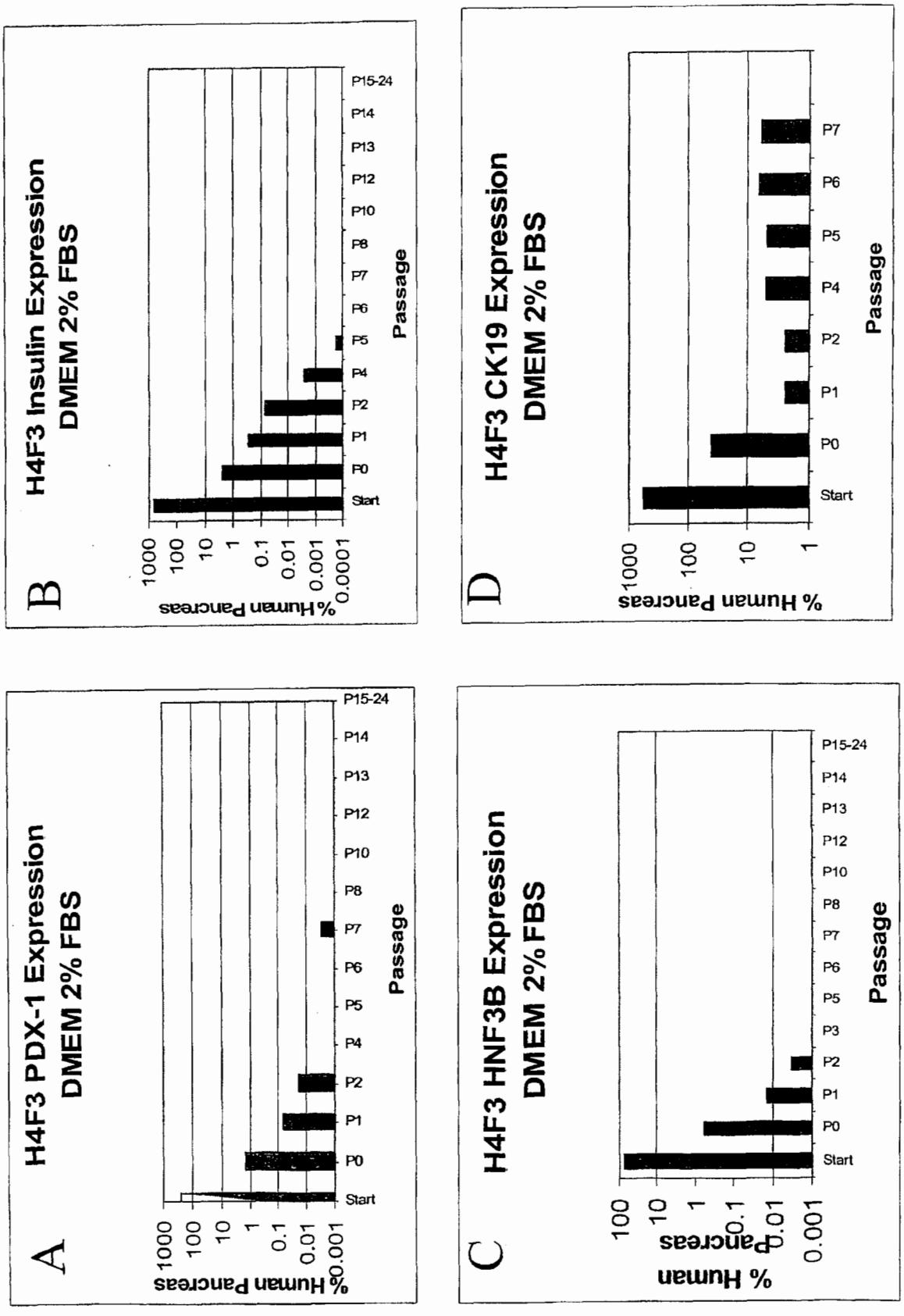
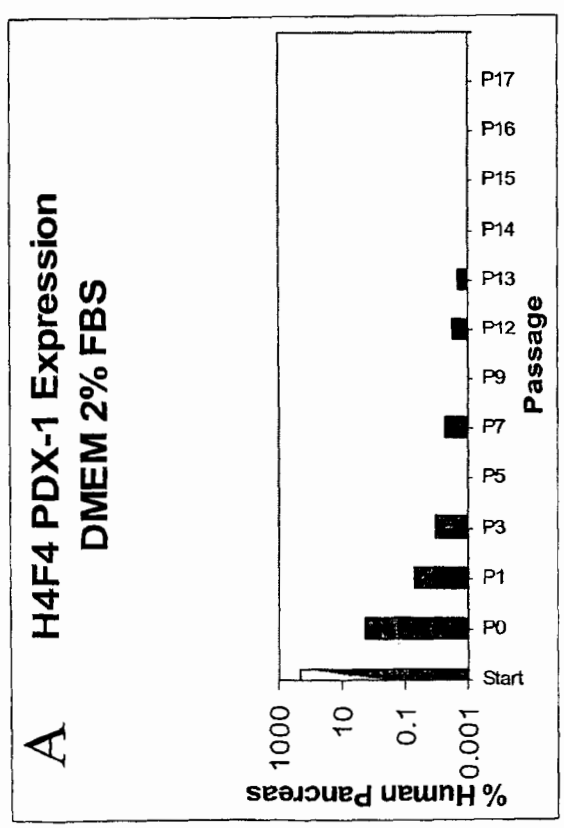
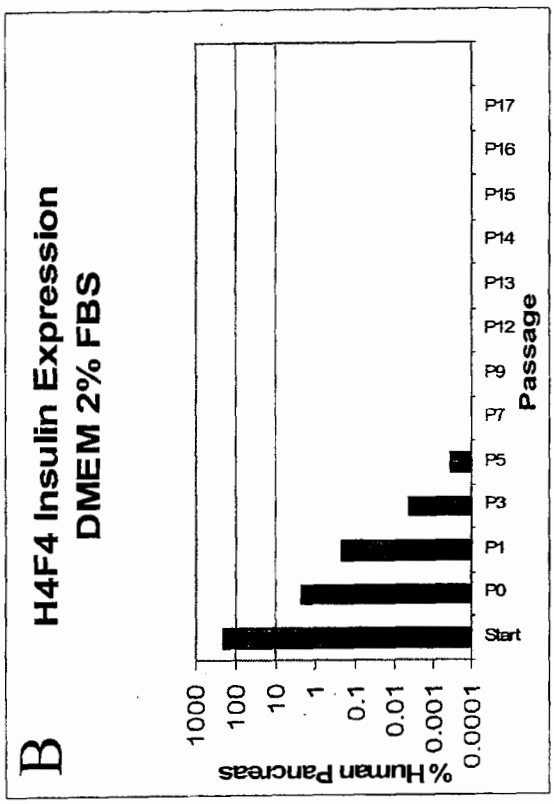
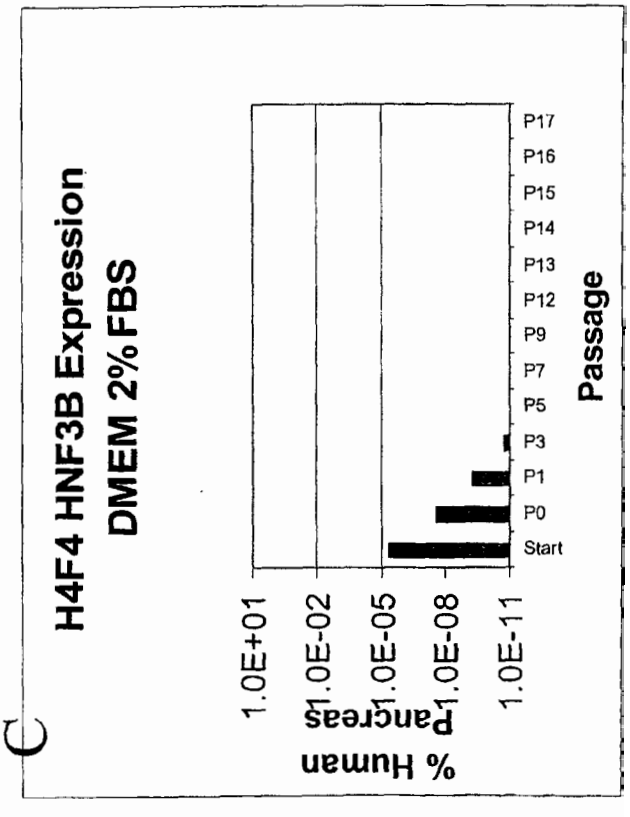

A
H4F3 PDX-1 Expression
DMEM 2% FBS
% Human Pancreas
1000
100
10
1
0.1
0.01
0.001
Start
P0
P1
P2
P4
P5
P6
P7
P8
P10
P12
P13
P14
P15-24
Passage
B
H4F3 Insulin Expression
DMEM 2% FBS
% Human Pancreas
1000
100
10
1
0.1
0.01
0.001
0.0001
Start
P0
P1
P2
P4
P5
P6
P7
P8
P10
P12
P13
P14
P15-24
Passage
C
H4F3 HNF3B Expression
DMEM 2% FBS
% Human Pancreas
100
10
1
0.1
0.01
0.001
Start
P0
P1
P2
P3
P5
P6
P7
P8
P10
P12
P13
P14
P15-24
Passage
D
H4F3 CK19 Expression
DMEM 2% FBS
% Human Pancreas
1000
100
10
1
Start
P0
P1
P2
P4
P5
P6
P7
Passage

Figure 8


A
H4F4 PDX-1 Expression
DMEM 2% FBS
% Human Pancreas
1000
10
0.1
0.001
Start
P0
P1
P3
P5
P7
P9
P12
P13
P14
P15
P16
P17
Passage


B
H4F4 Insulin Expression
DMEM 2% FBS
% Human Pancreas
1000
100
10
1
0.1
0.01
0.001
0.0001
Start
P0
P1
P3
P5
P7
P9
P12
P13
P14
P15
P16
P17
Passage


C
H4F4 HNF3B Expression
DMEM 2% FBS
% Human Pancreas
1.0E+01
1.0E-02
1.0E-05
1.0E-08
1.0E-11
Start
P0
P1
P3
P5
P7
P9
P12
P13
P14
P15
P16
P17
Passage

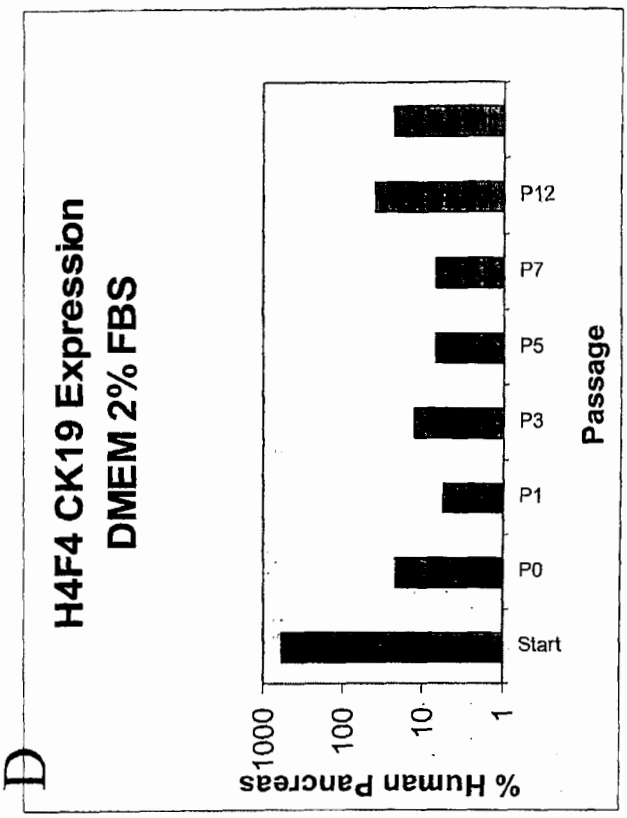

D
H4F4 CK19 Expression
DMEM 2% FBS
% Human Pancreas
1000
100
10
1
Start
P0
P1
P3
P5
P7
P12
Passage

Figure 9

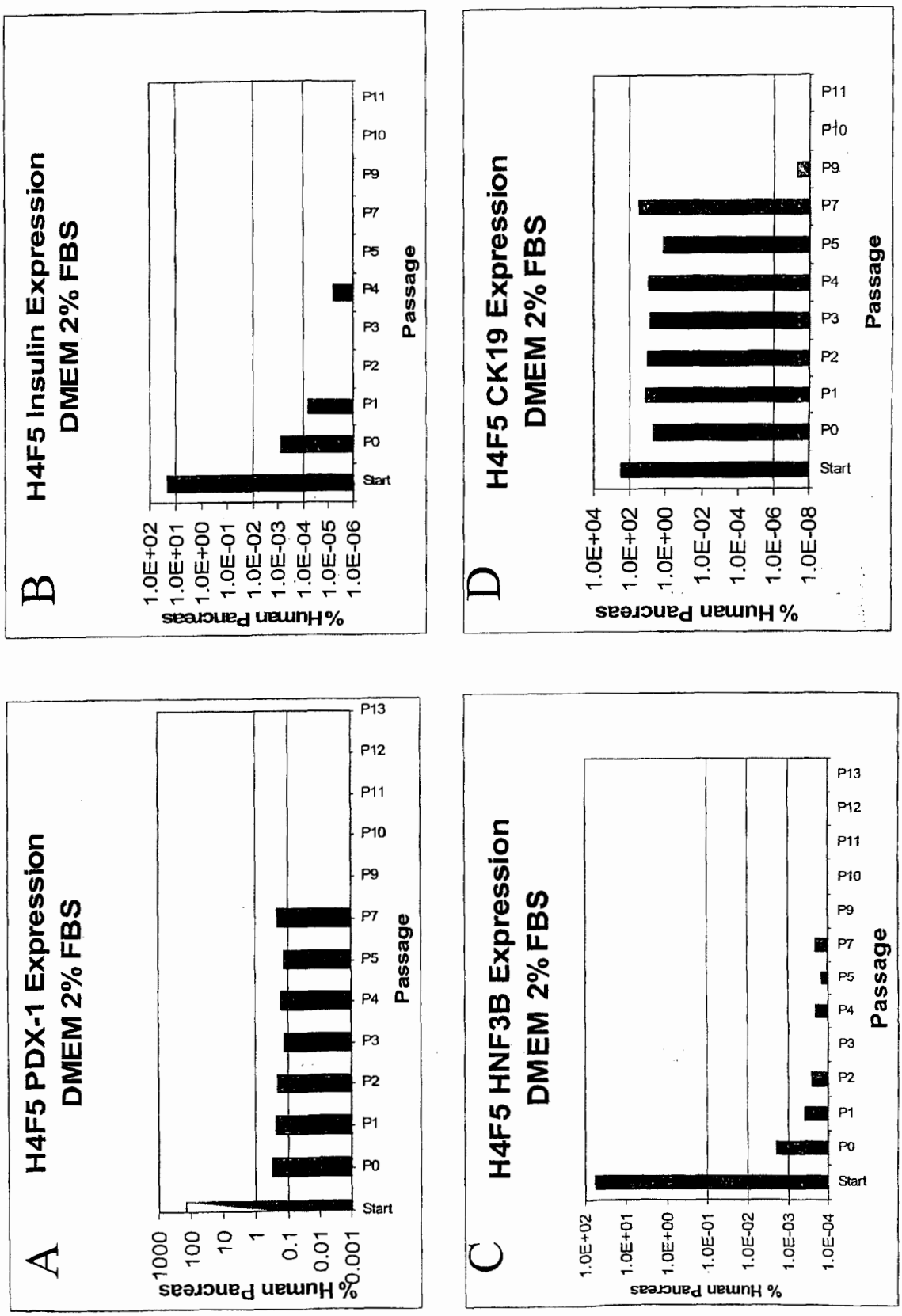

A
H4F5 PDX-1 Expression
DMEM 2% FBS
% Human Pancreas
1000
100
10
1
0.1
0.01
0.001
Start
P0
P1
P2
P3
P4
P5
P7
P9
P10
P11
P12
P13
Passage
B
H4F5 Insulin Expression
DMEM 2% FBS
% Human Pancreas
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
1.0E-05
1.0E-06
Start
P0
P1
P2
P3
P4
P5
P7
P9
P10
P11
Passage
C
H4F5 HNF3B Expression
DMEM 2% FBS
% Human Pancreas
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
Start
P0
P1
P2
P3
P4
P5
P7
P9
P10
P11
P12
P13
Passage
D
H4F5 CK19 Expression
DMEM 2% FBS
% Human Pancreas
1.0E+04
1.0E+02
1.0E+00
1.0E-02
1.0E-04
1.0E-06
1.0E-08
Start
P0
P1
P2
P3
P4
P5
P7
P9
P10
P11
Passage

Figure 10

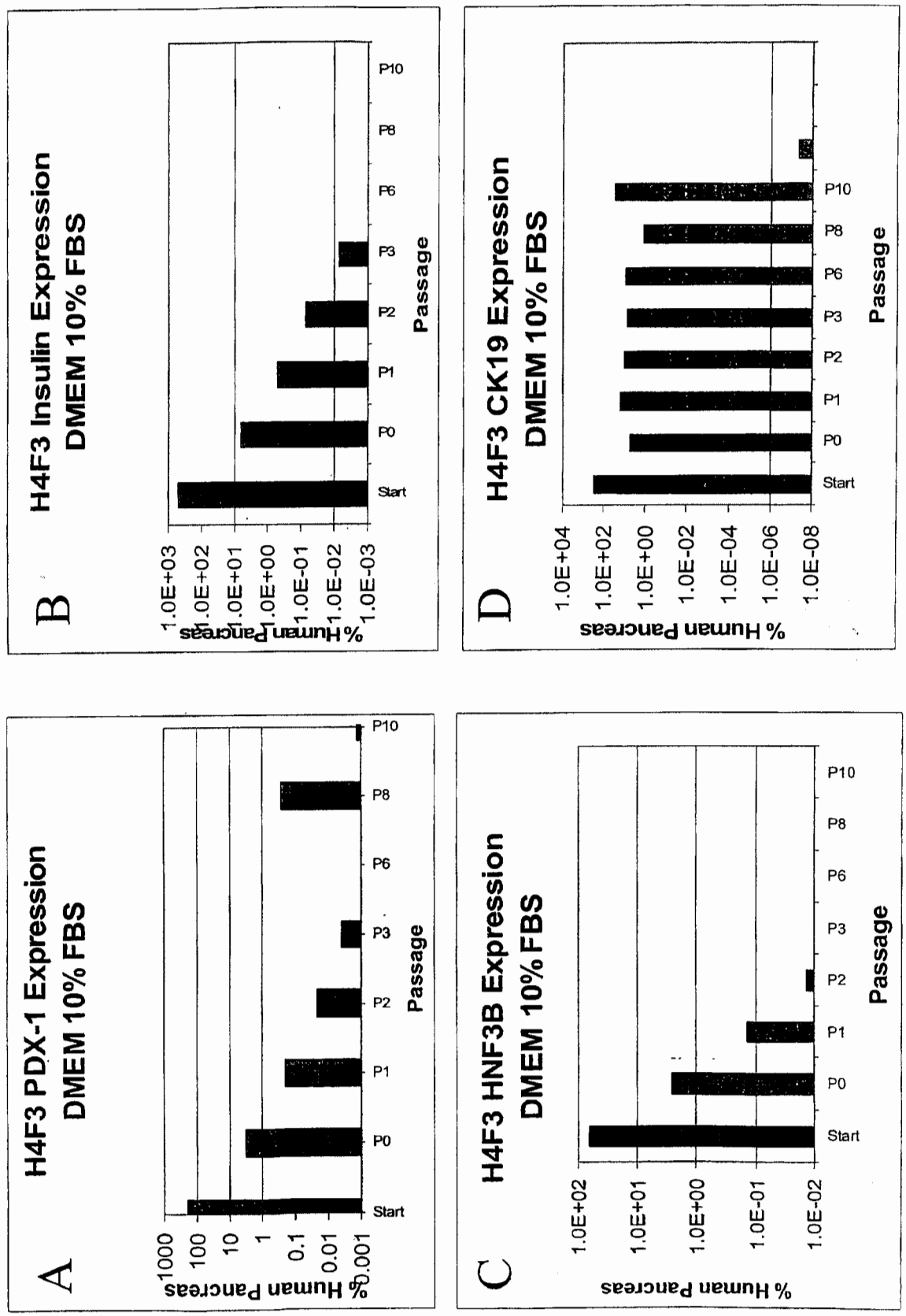

A
H4F3 PDX-1 Expression
DMEM 10% FBS
% Human Pancreas
1000
100
10
1
0.1
0.01
0.001
Start
P0
P1
P2
P3
P6
P8
P10
Passage
B
H4F3 Insulin Expression
DMEM 10% FBS
% Human Pancreas
1.0E+03
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
Start
P0
P1
P2
P3
P6
P8
P10
Passage
C
H4F3 HNF3B Expression
DMEM 10% FBS
% Human Pancreas
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
Start
P0
P1
P2
P3
P6
P8
P10
Passage
D
H4F3 CK19 Expression
DMEM 10% FBS
% Human Pancreas
1.0E+04
1.0E+02
1.0E+00
1.0E-02
1.0E-04
1.0E-06
1.0E-08
Start
P0
P1
P2
P3
P6
P8
P10
Passage

Figure 11

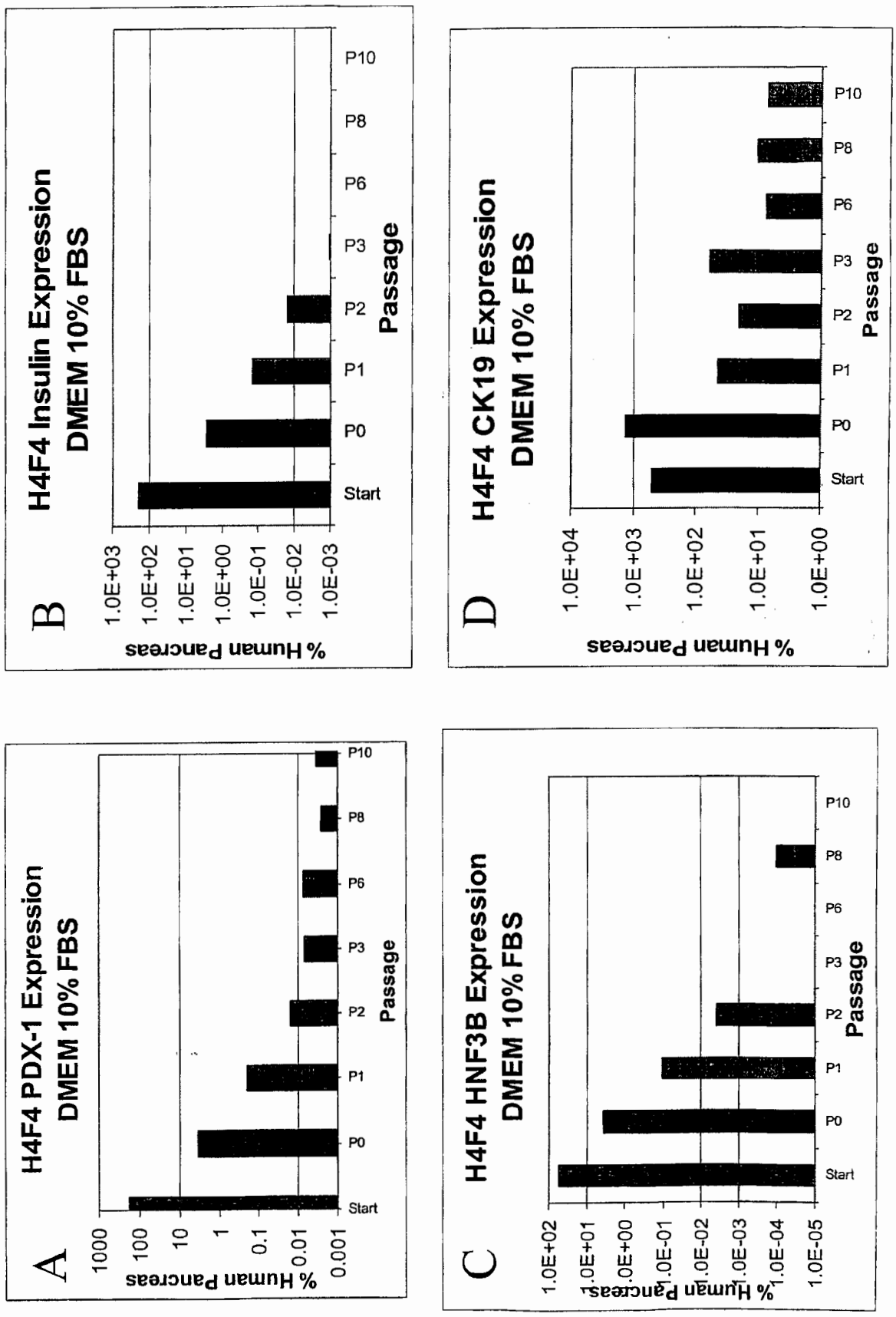

A
H4F4 PDX-1 Expression
DMEM 10% FBS
% Human Pancreas
1000
100
10
1
0.1
0.01
0.001
Start
P0
P1
P2
P3
P6
P8
P10
Passage
B
H4F4 Insulin Expression
DMEM 10% FBS
% Human Pancreas
1.0E+03
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
Start
P0
P1
P2
P3
P6
P8
P10
Passage
C
H4F4 HNF3B Expression
DMEM 10% FBS
% Human Pancreas
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
1.0E-05
Start
P0
P1
P2
P3
P6
P8
P10
Passage
D
H4F4 CK19 Expression
DMEM 10% FBS
% Human Pancreas
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
Start
P0
P1
P2
P3
P6
P8
P10
Passage

Figure 12

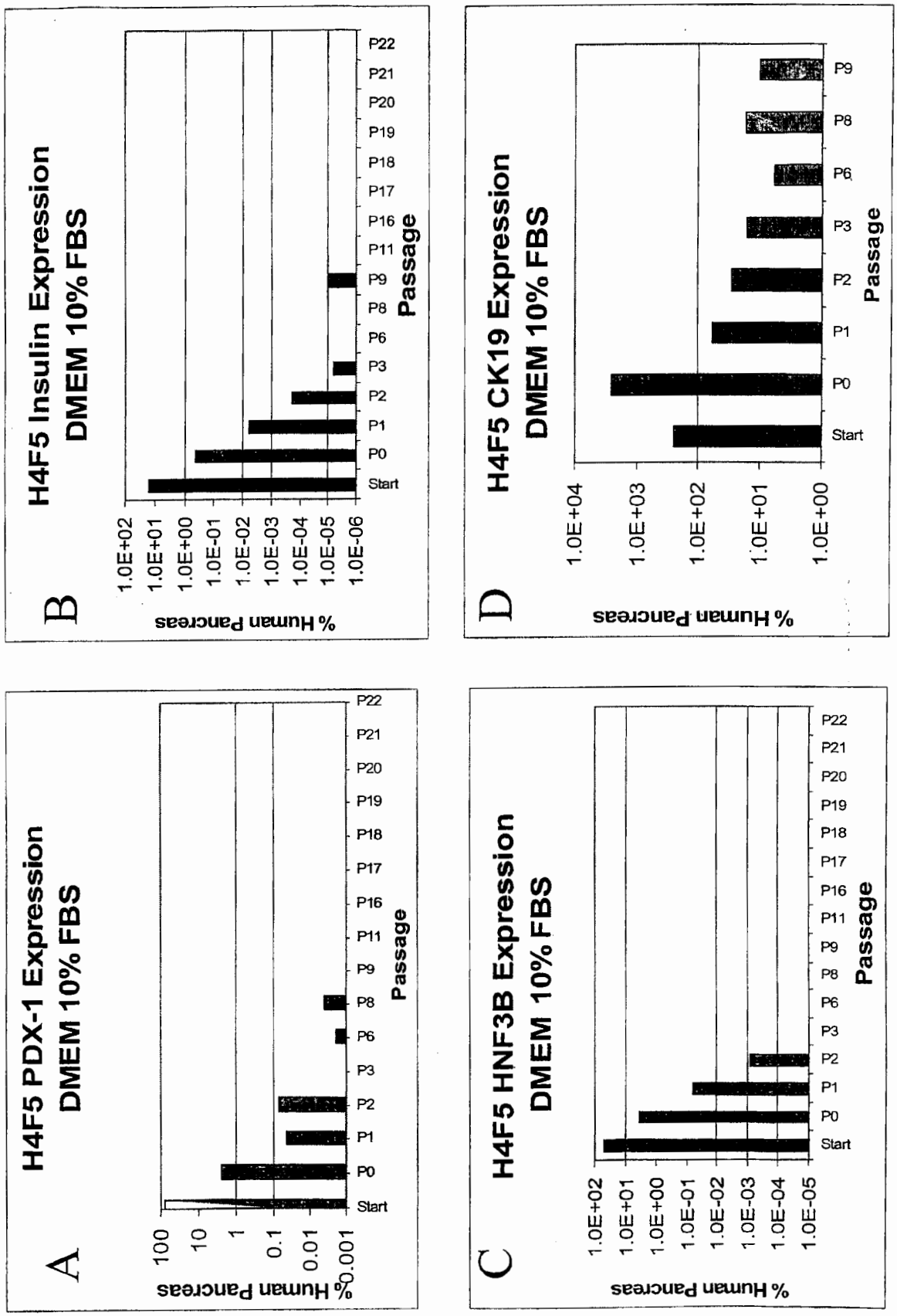

A
H4F5 PDX-1 Expression
DMEM 10% FBS
% Human Pancreas
100
10
1
0.1
0.01
0.001
Start
P0
P1
P2
P3
P6
P8
P9
P11
P16
P17
P18
P19
P20
P21
P22
Passage
B
H4F5 Insulin Expression
DMEM 10% FBS
% Human Pancreas
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
1.0E-05
1.0E-06
Start
P0
P1
P2
P3
P6
P8
P9
P11
P16
P17
P18
P19
P20
P21
P22
Passage
C
H4F5 HNF3B Expression
DMEM 10% FBS
% Human Pancreas
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
1.0E-05
Start
P0
P1
P2
P3
P6
P8
P9
P11
P16
P17
P18
P19
P20
P21
P22
Passage
D
H4F5 CK19 Expression
DMEM 10% FBS
% Human Pancreas
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
Start
P0
P1
P2
P3
P6
P8
P9
Passage

Figure 13

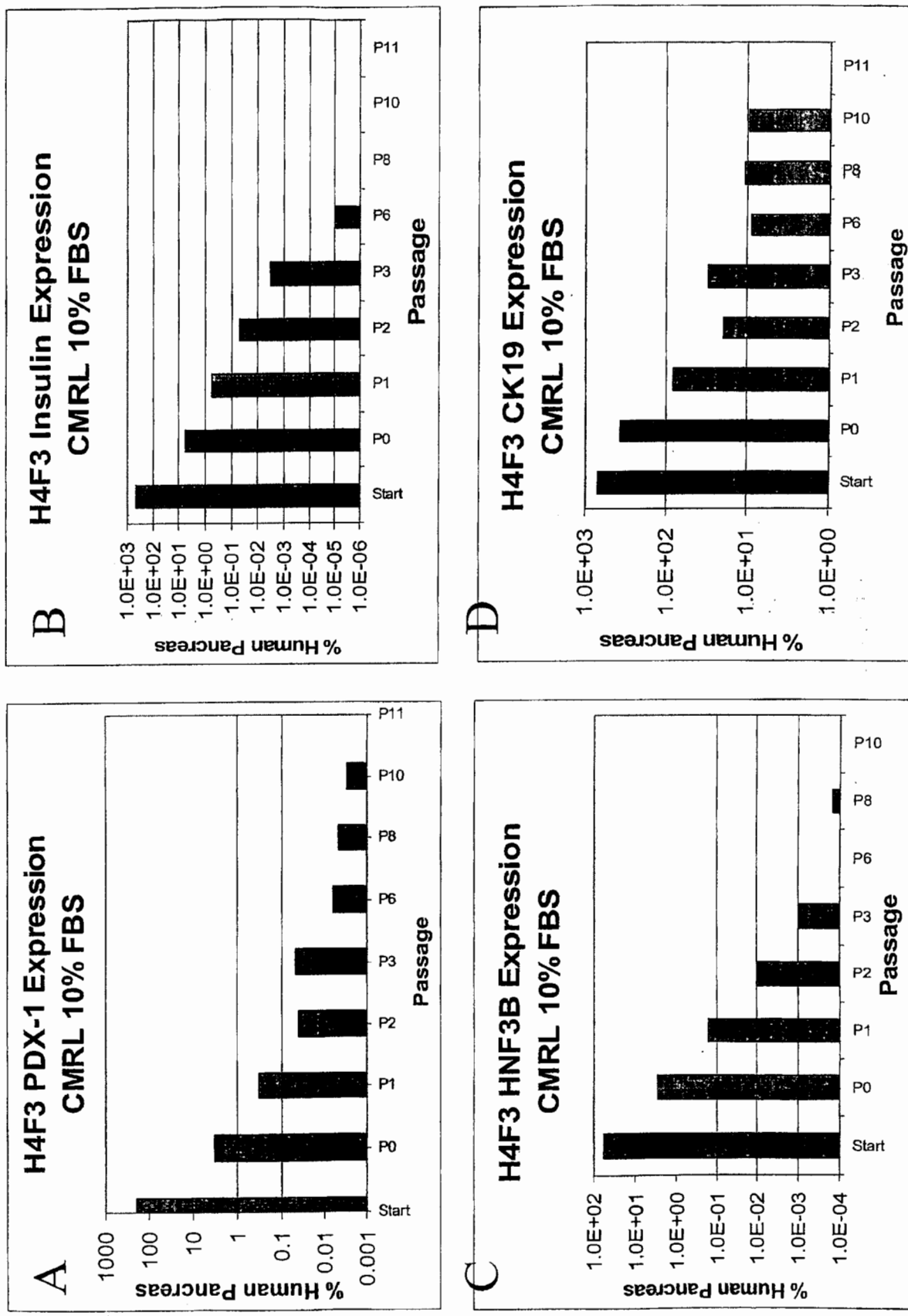

A
H4F3 PDX-1 Expression
CMRL 10% FBS
% Human Pancreas
1000
100
10
1
0.1
0.01
0.001
Start
P0
P1
P2
P3
P6
P8
P10
P11
Passage
B
H4F3 Insulin Expression
CMRL 10% FBS
% Human Pancreas
1.0E+03
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
1.0E-05
1.0E-06
Start
P0
P1
P2
P3
P6
P8
P10
P11
Passage
C
H4F3 HNF3B Expression
CMRL 10% FBS
% Human Pancreas
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
Start
P0
P1
P2
P3
P6
P8
P10
Passage
D
H4F3 CK19 Expression
CMRL 10% FBS
% Human Pancreas
1.0E+03
1.0E+02
1.0E+01
1.0E+00
Start
P0
P1
P2
P3
P6
P8
P10
P11
Passage

Figure 14

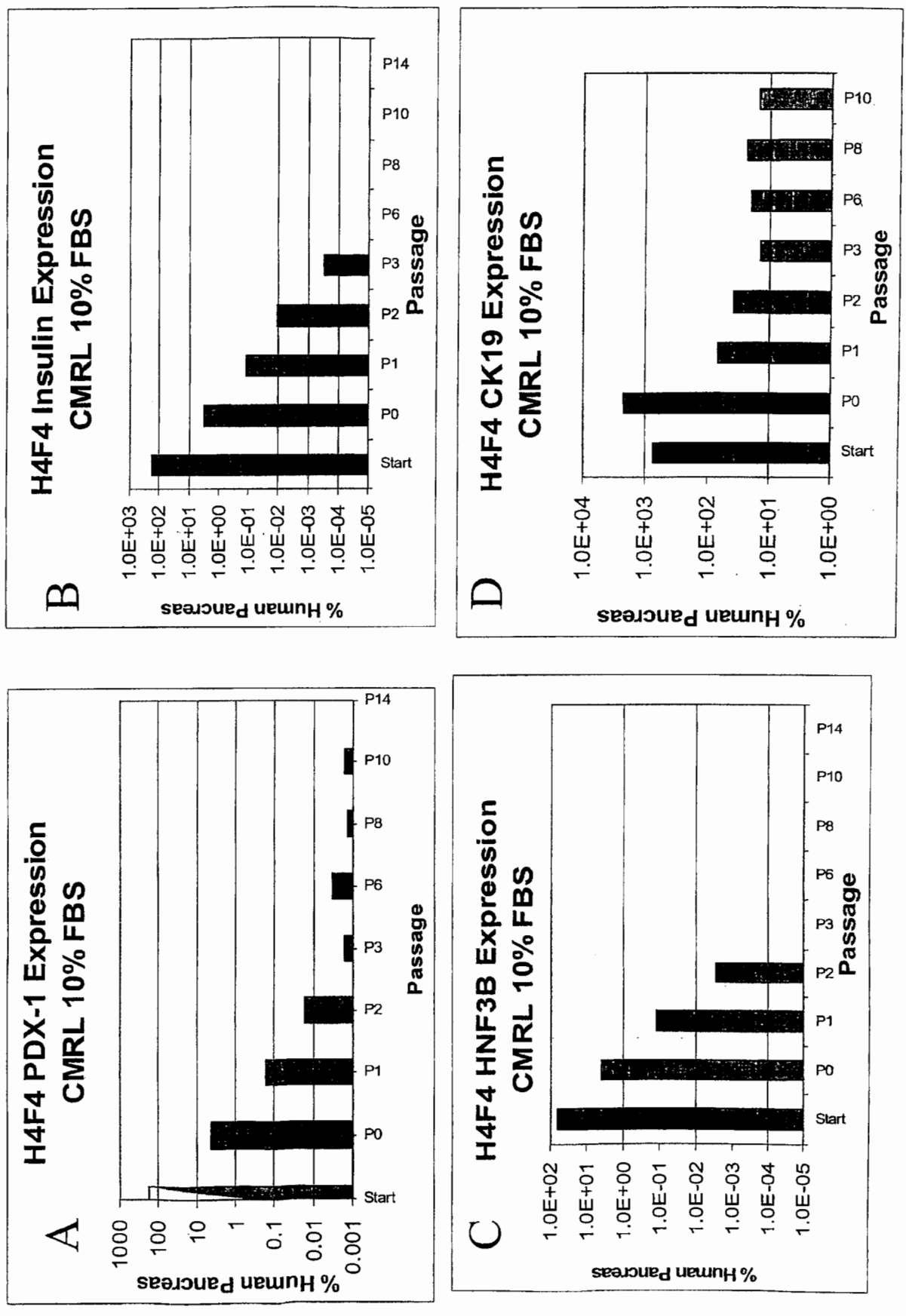

A
H4F4 PDX-1 Expression
CMRL 10% FBS
% Human Pancreas
1000
100
10
1
0.1
0.01
0.001
Start
P0
P1
P2
P3
P6
P8
P10
P14
Passage
B
H4F4 Insulin Expression
CMRL 10% FBS
% Human Pancreas
1.0E+03
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
1.0E-05
Start
P0
P1
P2
P3
P6
P8
P10
P14
Passage
C
H4F4 HNF3B Expression
CMRL 10% FBS
% Human Pancreas
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
1.0E-05
Start
P0
P1
P2
P3
P6
P8
P10
P14
Passage
D
H4F4 CK19 Expression
CMRL 10% FBS
% Human Pancreas
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
Start
P0
P1
P2
P3
P6
P8
P10
Passage

Figure 15

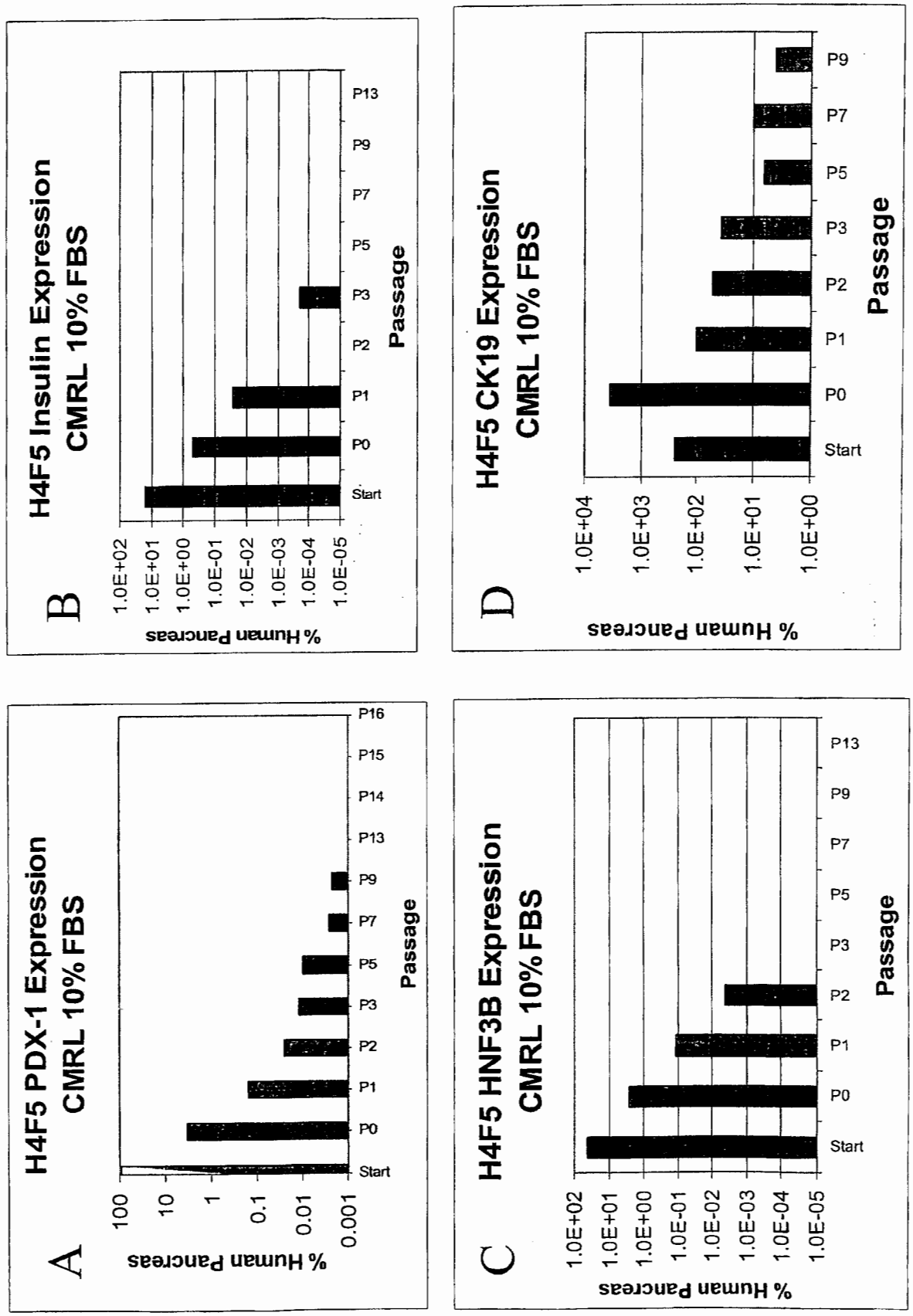

A
H4F5 PDX-1 Expression
CMRL 10% FBS
% Human Pancreas
100
10
1
0.1
0.01
0.001
Start
P0
P1
P2
P3
P5
P7
P9
P13
P14
P15
P16
Passage
B
H4F5 Insulin Expression
CMRL 10% FBS
% Human Pancreas
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
1.0E-05
Start
P0
P1
P2
P3
P5
P7
P9
P13
Passage
C
H4F5 HNF3B Expression
CMRL 10% FBS
% Human Pancreas
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
1.0E-05
Start
P0
P1
P2
P3
P5
P7
P9
P13
Passage
D
H4F5 CK19 Expression
CMRL 10% FBS
% Human Pancreas
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
Start
P0
P1
P2
P3
P5
P7
P9
Passage

Figure 16

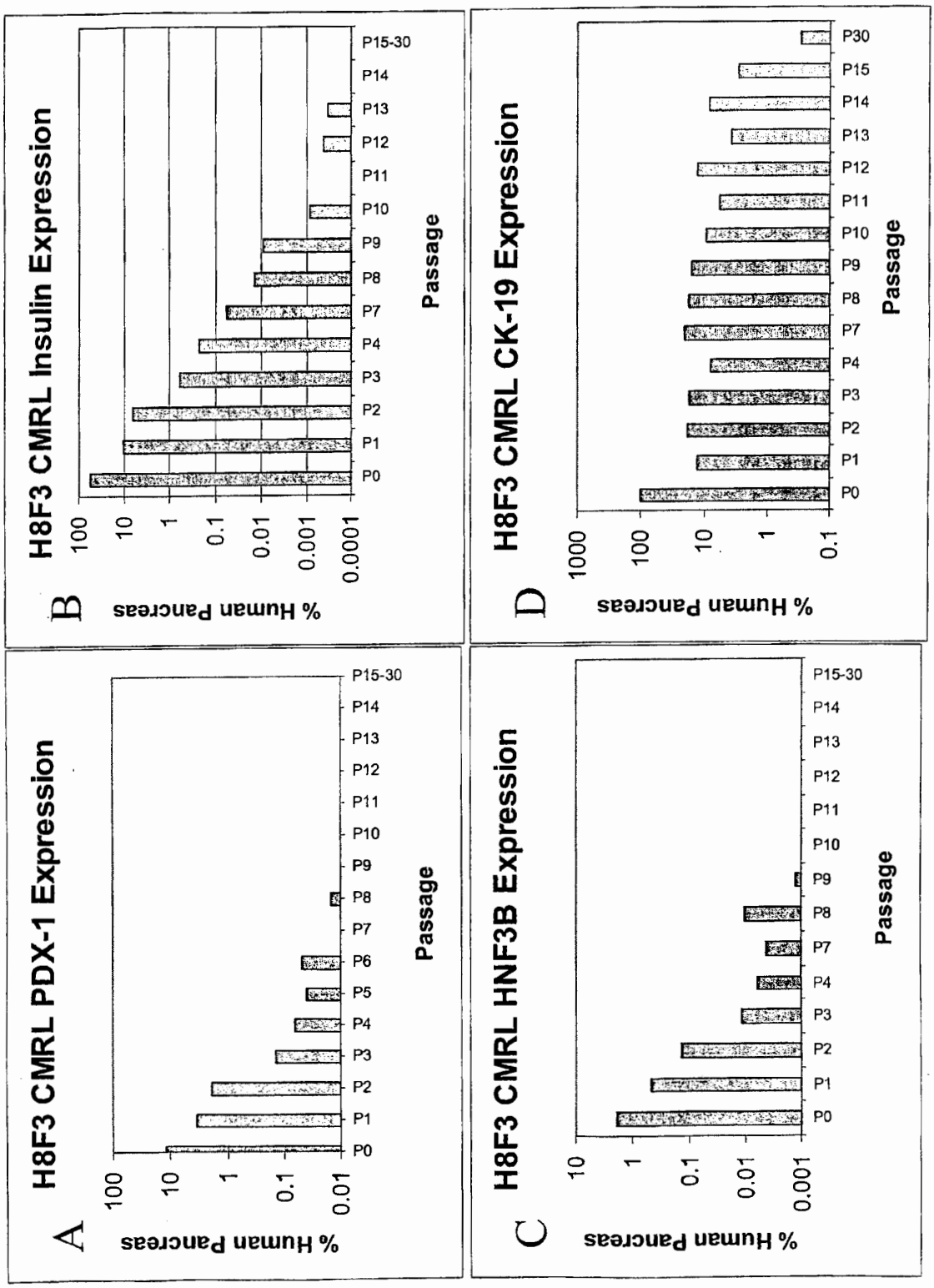

A
H8F3 CMRL PDX-1 Expression
% Human Pancreas
100
10
1
0.1
0.01
P0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
P13
P14
P15-30
Passage
B
H8F3 CMRL Insulin Expression
% Human Pancreas
100
10
1
0.1
0.01
0.001
0.0001
P0
P1
P2
P3
P4
P7
P8
P9
P10
P11
P12
P13
P14
P15-30
Passage
C
H8F3 CMRL HNF3B Expression
% Human Pancreas
10
1
0.1
0.01
0.001
P0
P1
P2
P3
P4
P7
P8
P9
P10
P11
P12
P13
P14
P15-30
Passage
D
H8F3 CMRL CK-19 Expression
% Human Pancreas
1000
100
10
1
0.1
P0
P1
P2
P3
P4
P7
P8
P9
P10
P11
P12
P13
P14
P15
P30
Passage

Figure 17

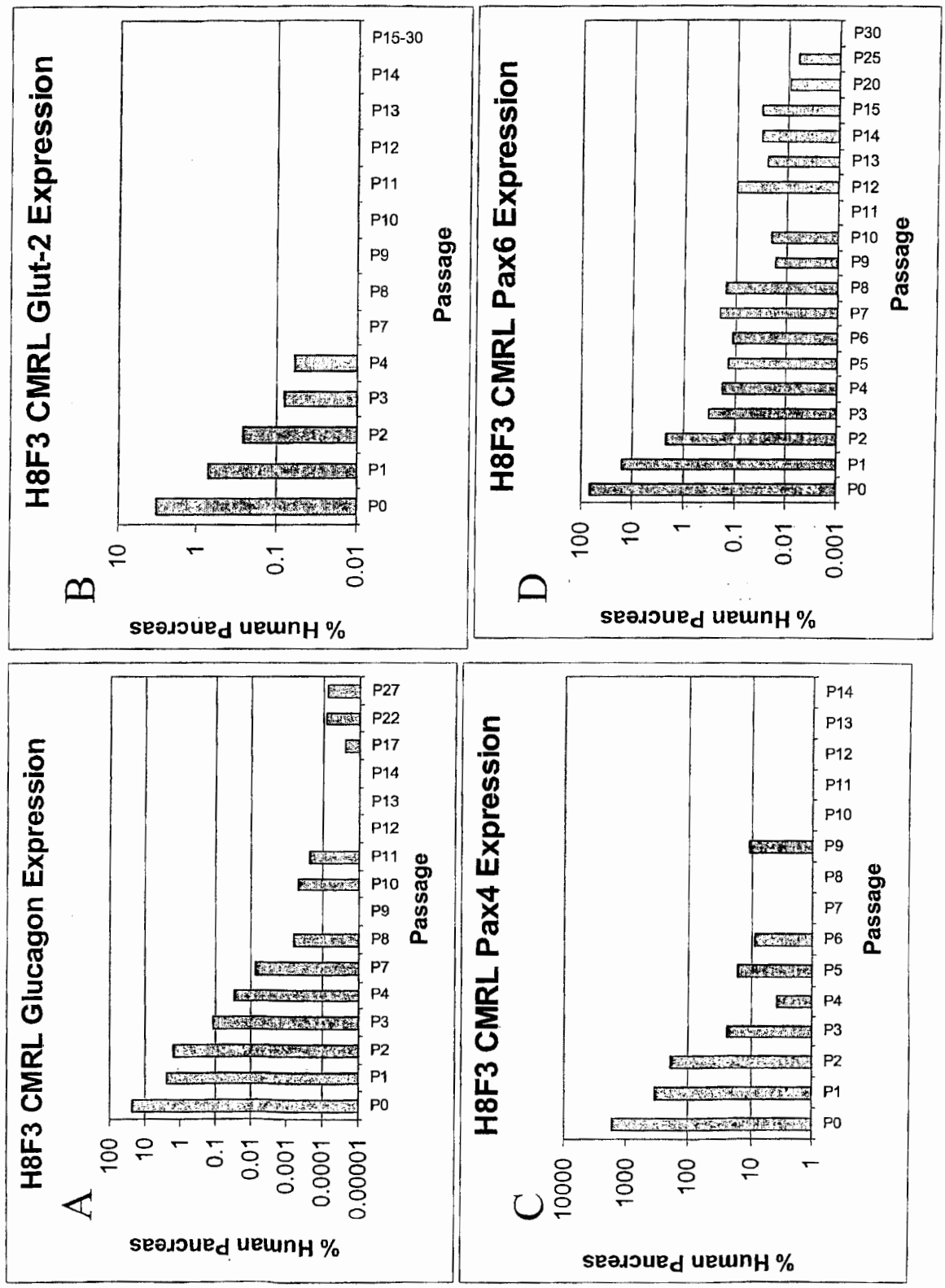

A
H8F3 CMRL Glucagon Expression
% Human Pancreas
100
10
1
0.1
0.01
0.001
0.0001
0.00001
P0
P1
P2
P3
P4
P7
P8
P9
P10
P11
P12
P13
P14
P17
P22
P27
Passage
B
H8F3 CMRL Glut-2 Expression
% Human Pancreas
10
1
0.1
0.01
P0
P1
P2
P3
P4
P7
P8
P9
P10
P11
P12
P13
P14
P15-30
Passage
C
H8F3 CMRL Pax4 Expression
% Human Pancreas
10000
1000
100
10
1
P0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
P13
P14
Passage
D
H8F3 CMRL Pax6 Expression
% Human Pancreas
100
10
1
0.1
0.01
0.001
P0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
P13
P14
P15
P20
P25
P30
Passage

Figure 18

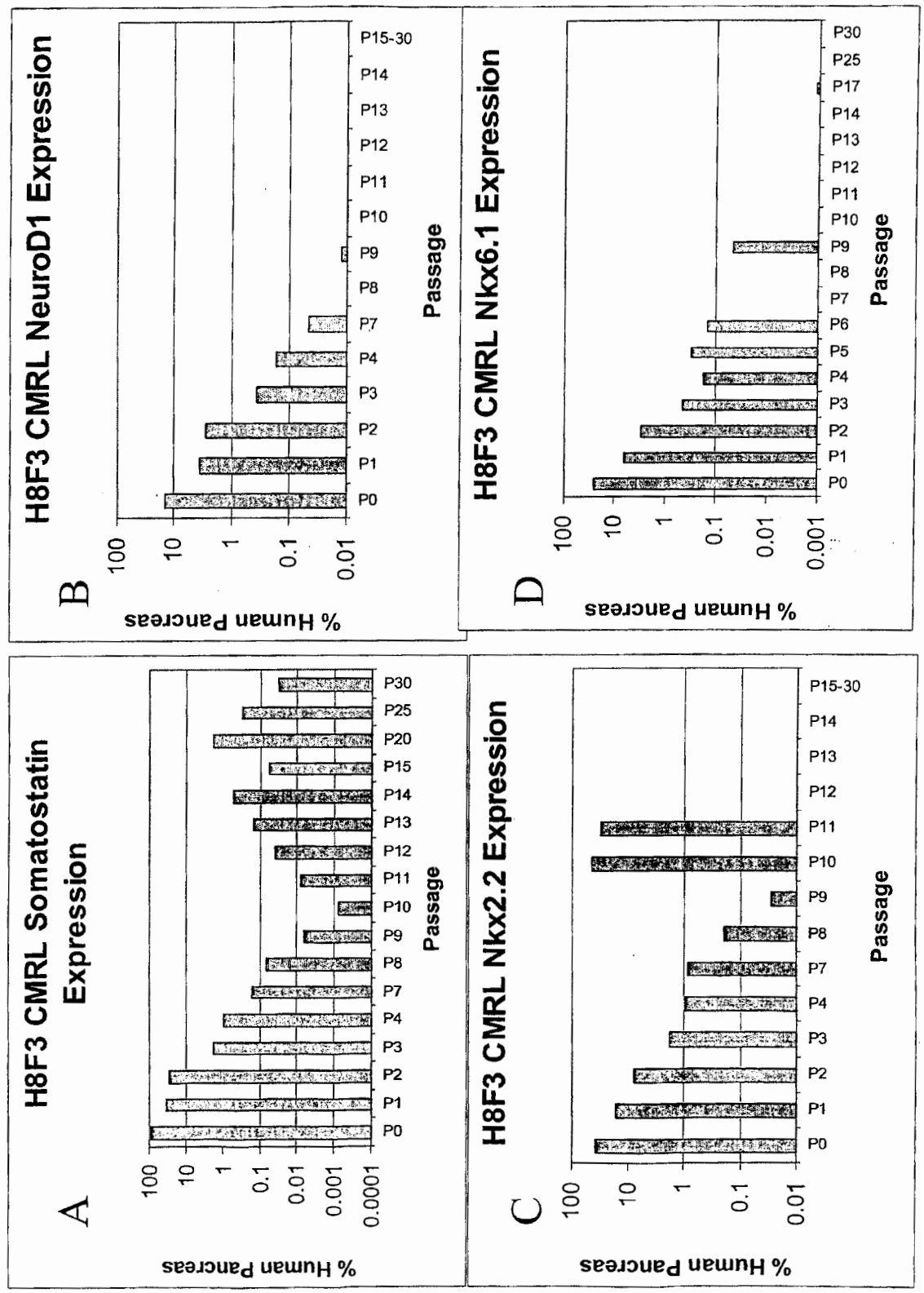

A
H8F3 CMRL Somatostatin Expression
% Human Pancreas
100
10
1
0.1
0.01
0.001
0.0001
P0
P1
P2
P3
P4
P7
P8
P9
P10
P11
P12
P13
P14
P15
P20
P25
P30
Passage
B
H8F3 CMRL NeuroD1 Expression
% Human Pancreas
100
10
1
0.1
0.01
P0
P1
P2
P3
P4
P7
P8
P9
P10
P11
P12
P13
P14
P15-30
Passage
C
H8F3 CMRL Nkx2.2 Expression
% Human Pancreas
100
10
1
0.1
0.01
P0
P1
P2
P3
P4
P7
P8
P9
P10
P11
P12
P13
P14
P15-30
Passage
D
H8F3 CMRL Nkx6.1 Expression
% Human Pancreas
100
10
1
0.1
0.01
0.001
P0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
P13
P14
P17
P25
P30
Passage

Figure 19

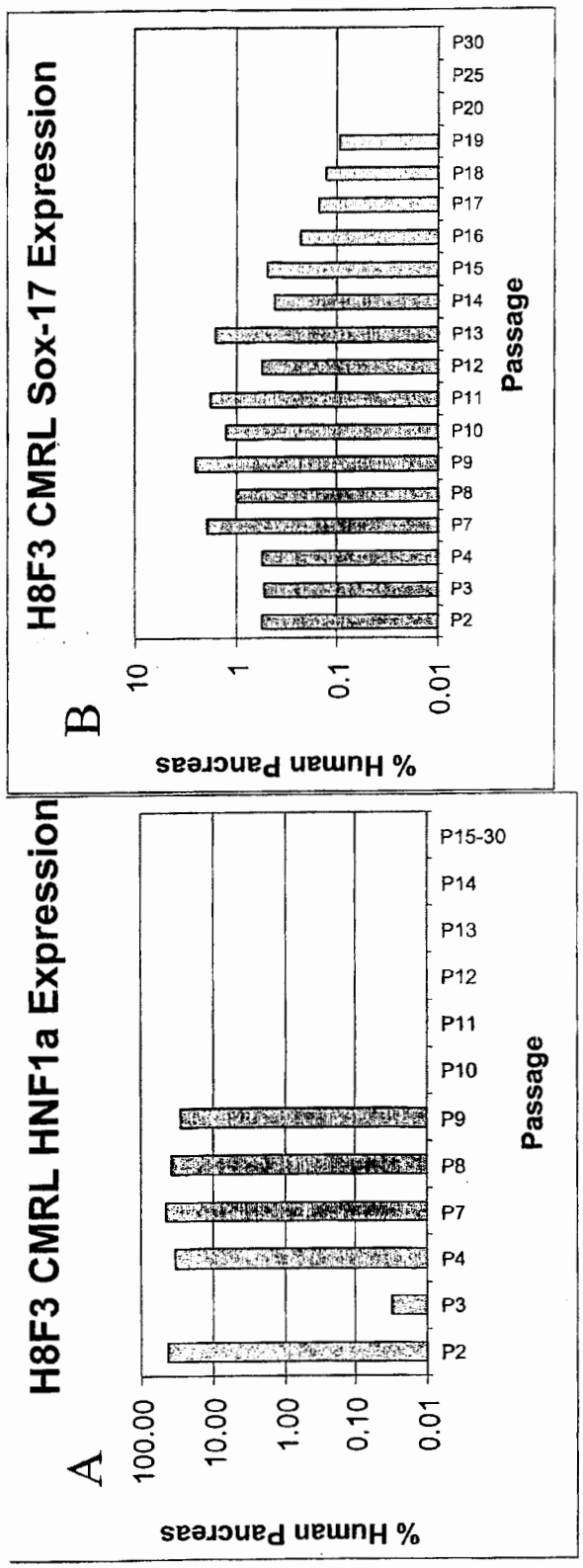

A
H8F3 CMRL HNF1a Expression
% Human Pancreas
100.00
10.00
1.00
0.10
0.01
P2
P3
P4
P7
P8
P9
P10
P11
P12
P13
P14
P15-30
Passage
B
H8F3 CMRL Sox-17 Expression
% Human Pancreas
10
1
0.1
0.01
P2
P3
P4
P7
P8
P9
P10
P11
P12
P13
P14
P15
P16
P17
P18
P19
P20
P25
P30
Passage

Figure 20

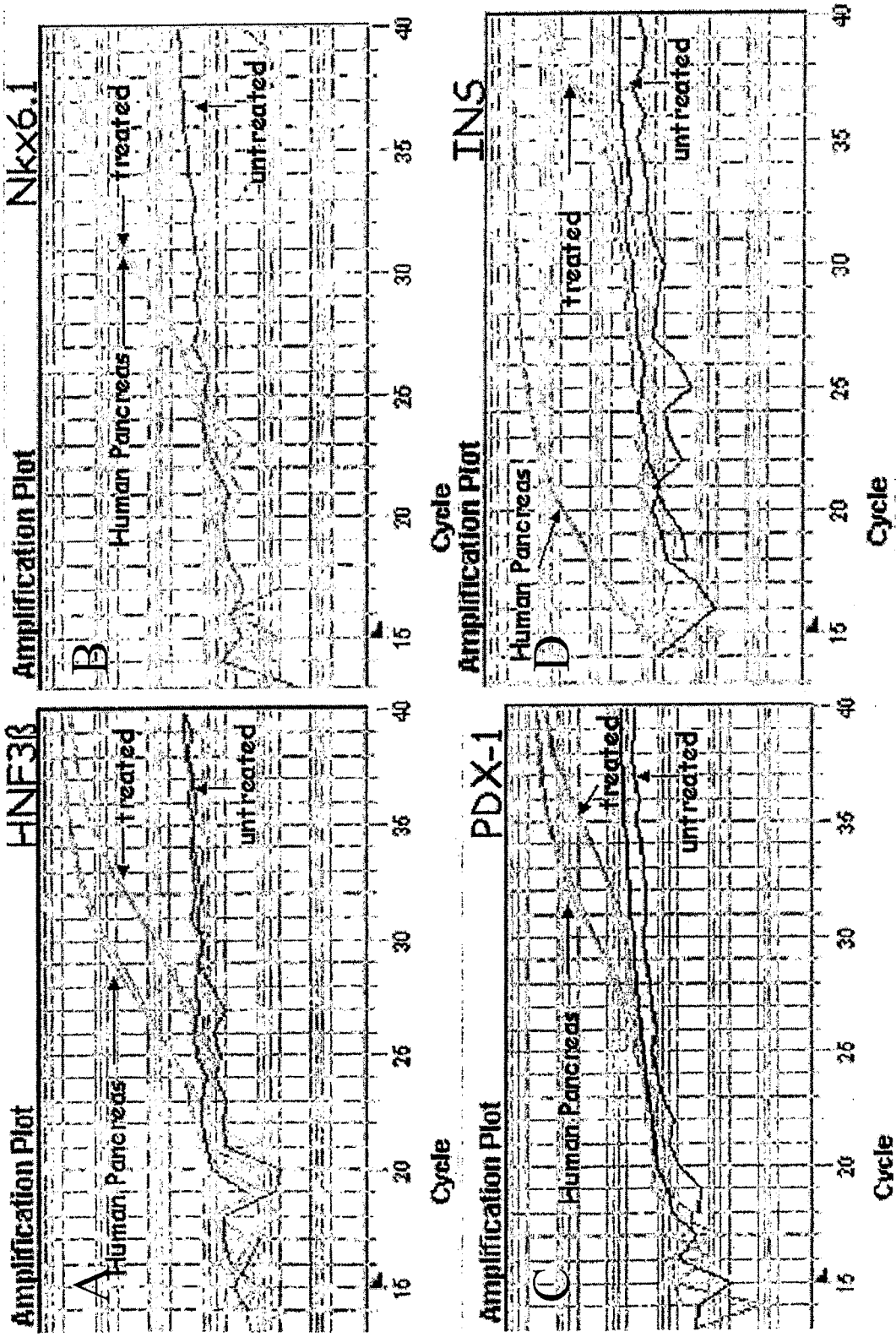

A
HNF3β
Amplification Plot
Human Pancreas
treated
untreated
Cycle
B
Nkx6.1
Amplification Plot
Human Pancreas
treated
untreated
Cycle
C
PDX-1
Amplification Plot
Human Pancreas
treated
untreated
Cycle
D
INS
Amplification Plot
Human Pancreas
treated
untreated
Cycle

Figure 21

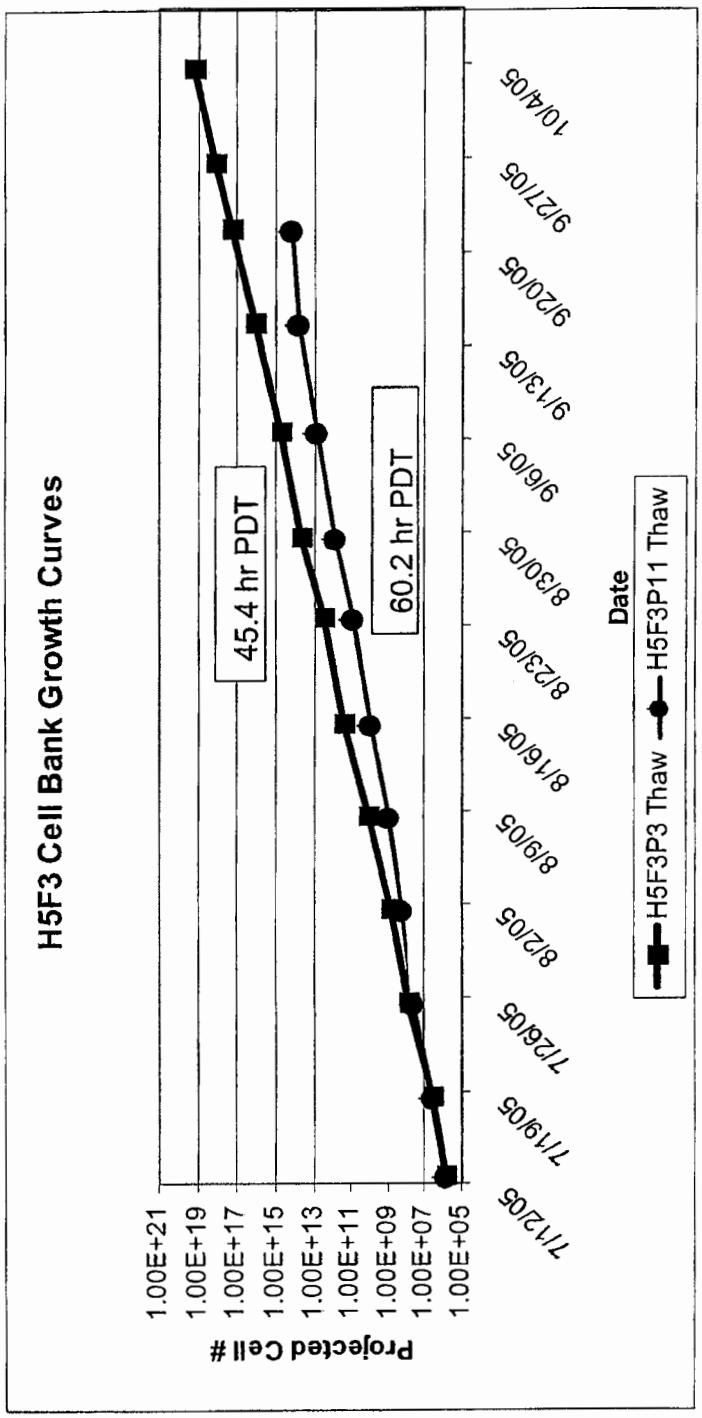

H5F3 Cell Bank Growth Curves
Projected Cell #
1.00E+21
1.00E+19
1.00E+17
1.00E+15
1.00E+13
1.00E+11
1.00E+09
1.00E+07
1.00E+05
7/12/05
7/19/05
7/26/05
8/2/05
8/9/05
8/16/05
8/23/05
8/30/05
9/6/05
9/13/05
9/20/05
9/27/05
10/4/05
45.4 hr PDT
60.2 hr PDT
Date
H5F3P3 Thaw
H5F3P11 Thaw

Figure 22

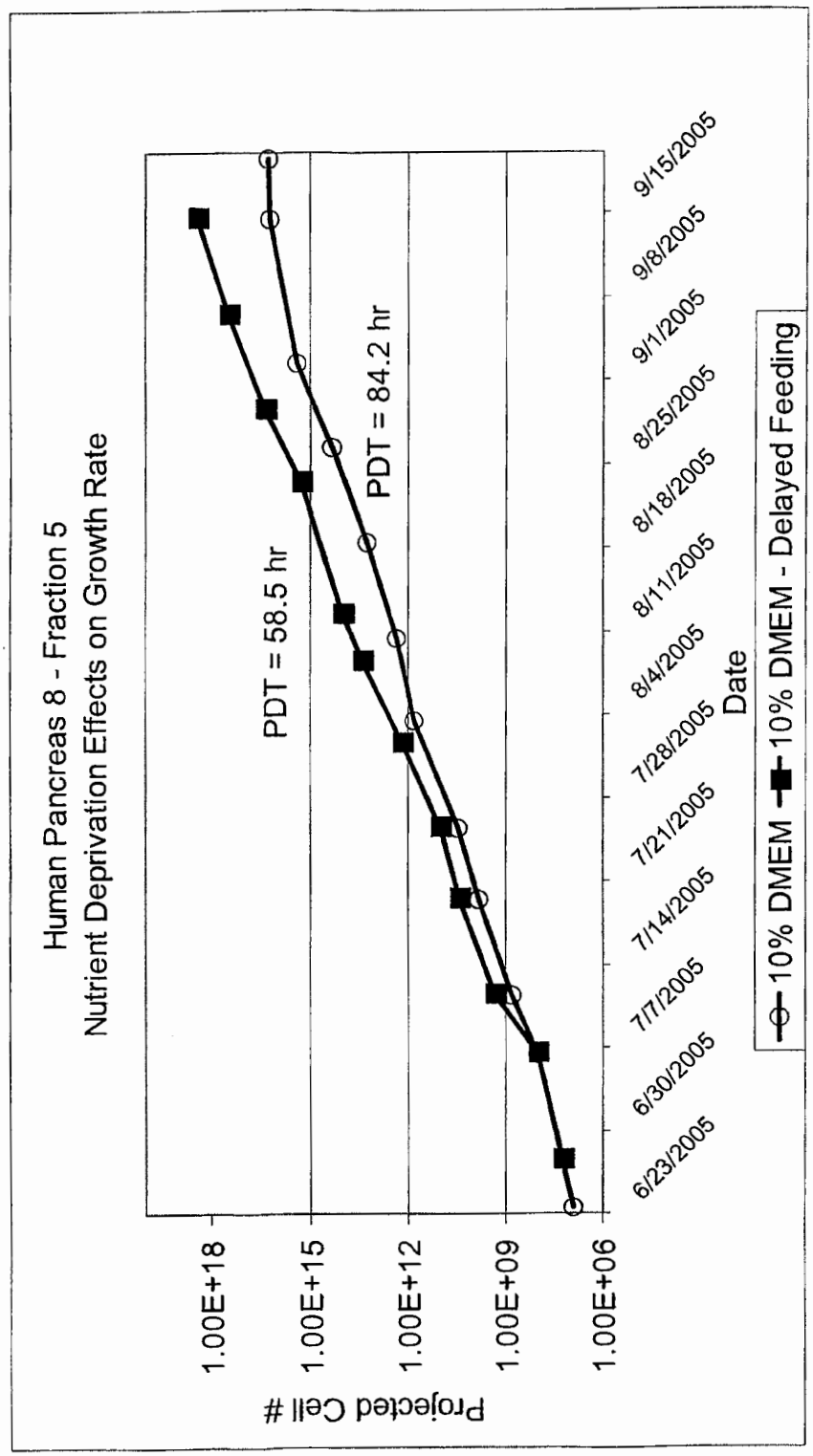

Human Pancreas 8 - Fraction 5
Nutrient Deprivation Effects on Growth Rate
PDT = 58.5 hr
PDT = 84.2 hr
Projected Cell #
1.00E+18
1.00E+15
1.00E+12
1.00E+09
1.00E+06
Date
6/23/2005
6/30/2005
7/7/2005
7/14/2005
7/21/2005
7/28/2005
8/4/2005
8/11/2005
8/18/2005
8/25/2005
9/1/2005
9/8/2005
9/15/2005
10% DMEM
10% DMEM - Delayed Feeding



Figure 23



Figure 24



Figure 25

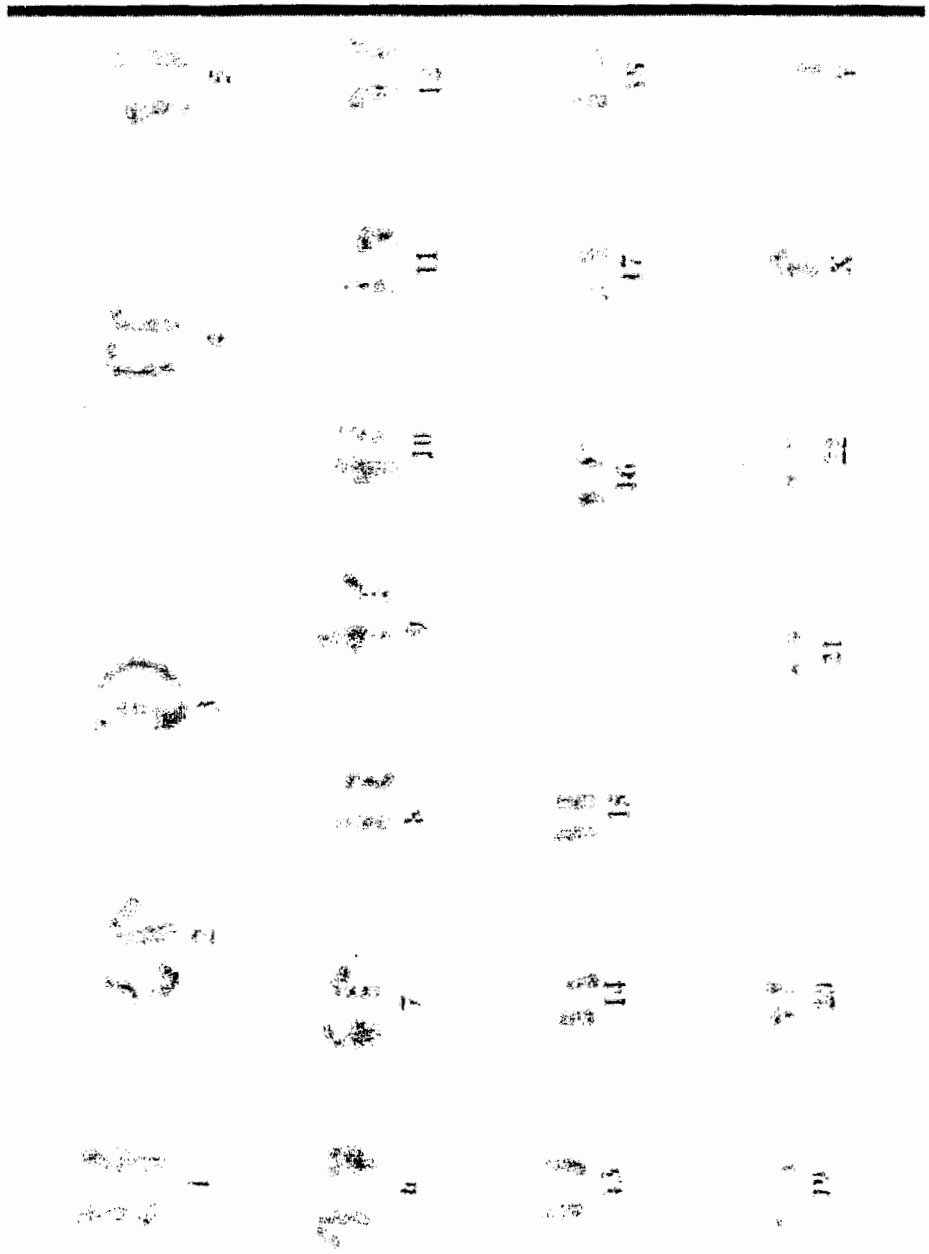


Figure 26



Figure 27

Figure 28

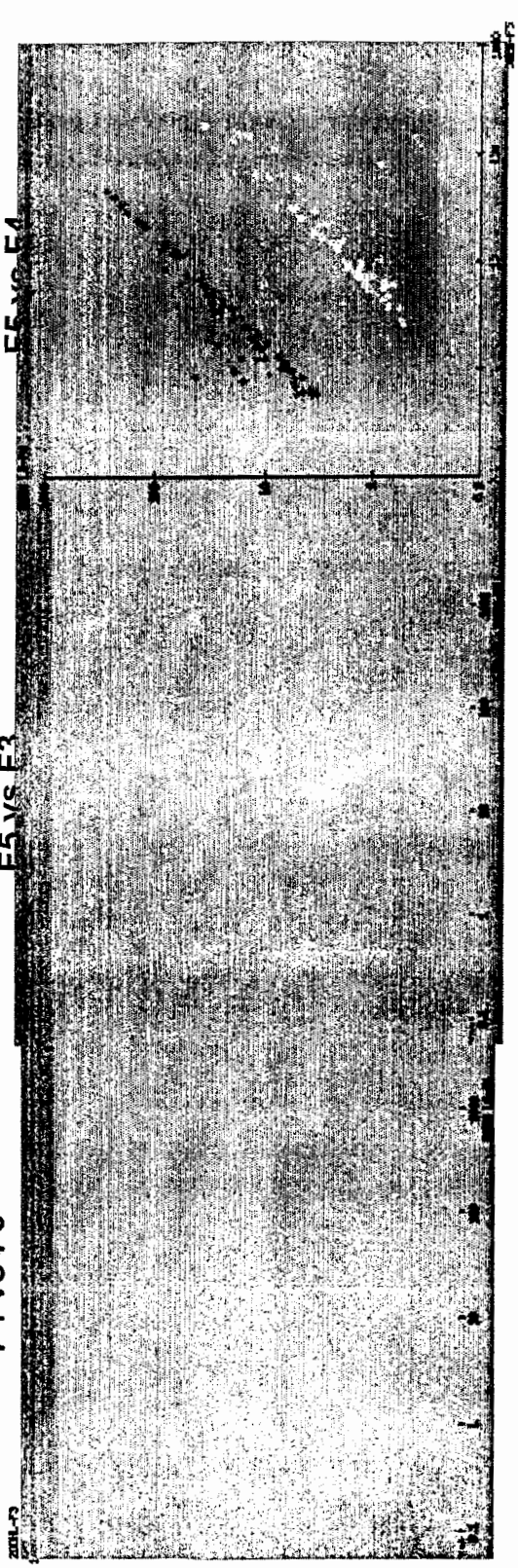
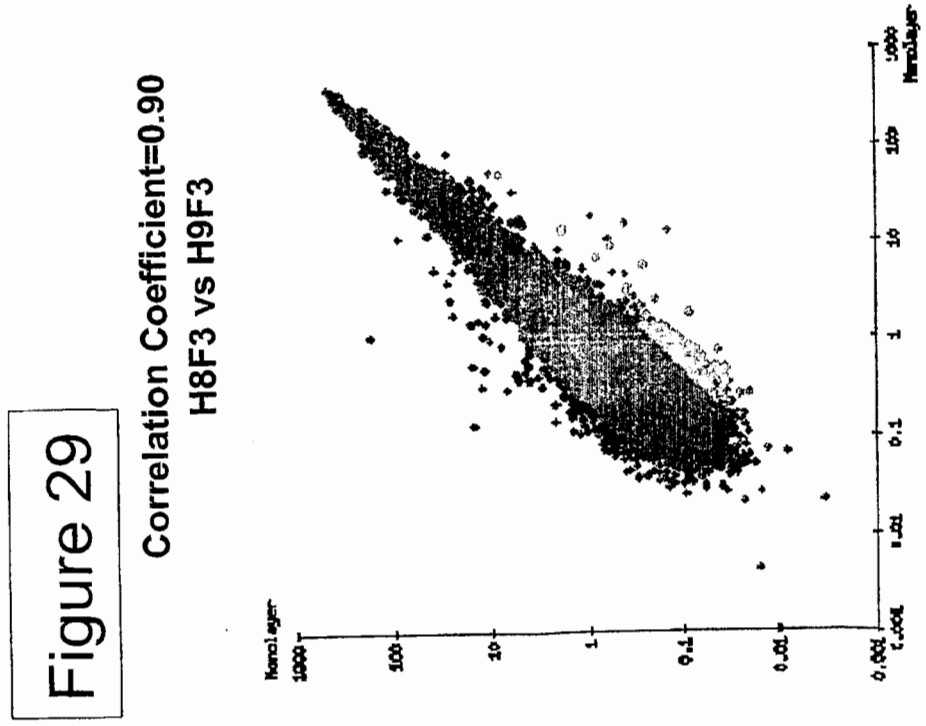

Correlation Coefficient=0.96
F4 vs F3
Correlation Coefficient=0.93
F5 vs F3
Correlation Coefficient=0.94
F5 vs F4

Figure 29


Correlation Coefficient=0.90
H8F3 vs H9F3
Normalized
1000
100
10
1
0.1
0.01
0.001
0.0001
0.001
0.01
0.1
1
10
100
1000
Normalized