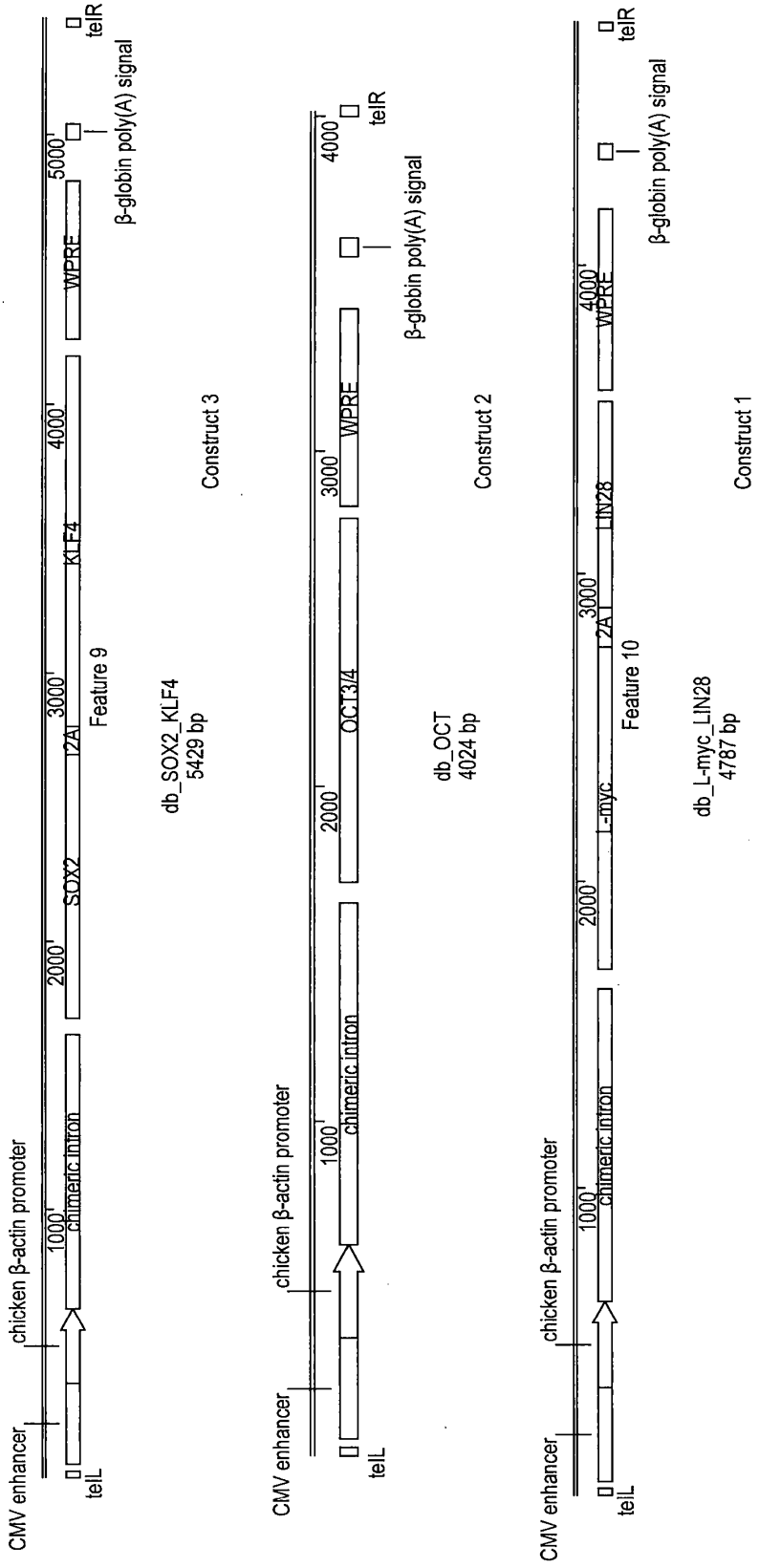

CMV enhancer
chicken β-actin promoter
chimeric intron
SOX2
2A
KLF4
WPRE
β-globin poly(A) signal
telL
telR
1000'
2000'
3000'
4000'
5000'
Feature 9
db_SOX2_KLF4
5429 bp
Construct 3
OCT3/4
Feature 10
db_OCT
4024 bp
Construct 2
L-myc
LIN28
db_L-myc_LIN28
4787 bp
Construct 1

Fig. 1

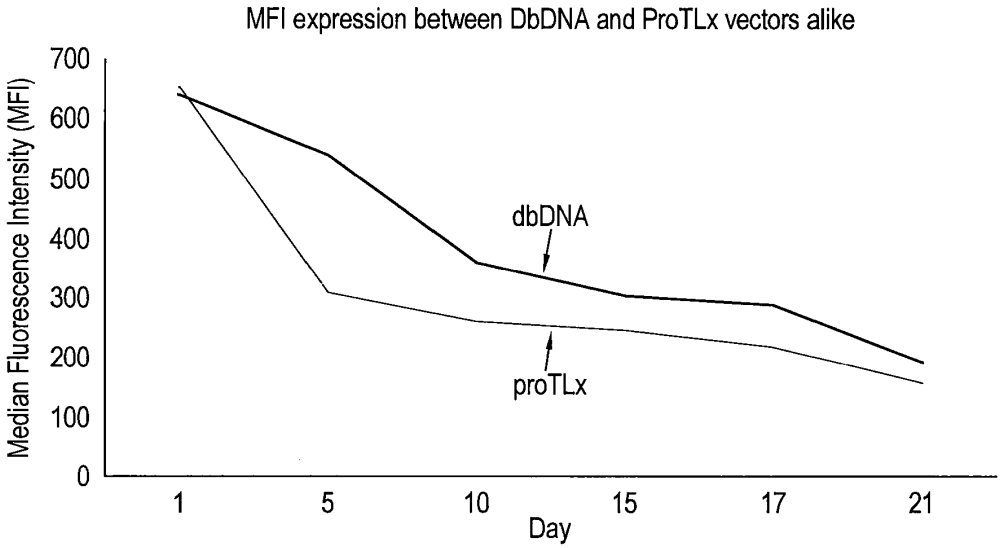

MFI expression between DbDNA and ProTLx vectors alike
Median Fluorescence Intensity (MFI)
700
600
500
400
300
200
100
0
dbDNA
proTLx
1
5
10
15
17
21
Day

Fig. 2A

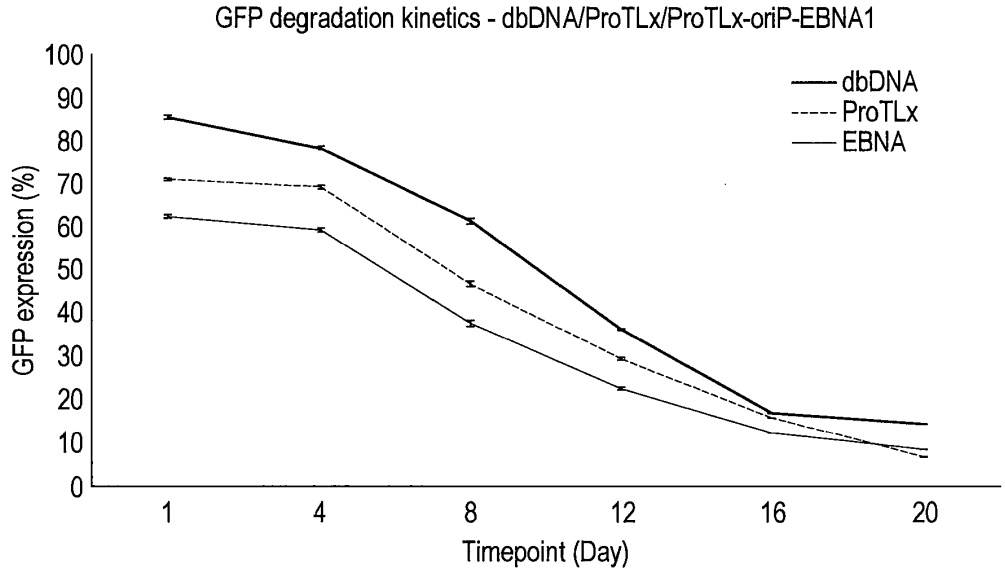

GFP degradation kinetics - dbDNA/ProTLx/ProTLx-oriP-EBNA1
GFP expression (%)
100
90
80
70
60
50
40
30
20
10
0
dbDNA
ProTLx
EBNA
1
4
8
12
16
20
Timepoint (Day)

Fig. 2B

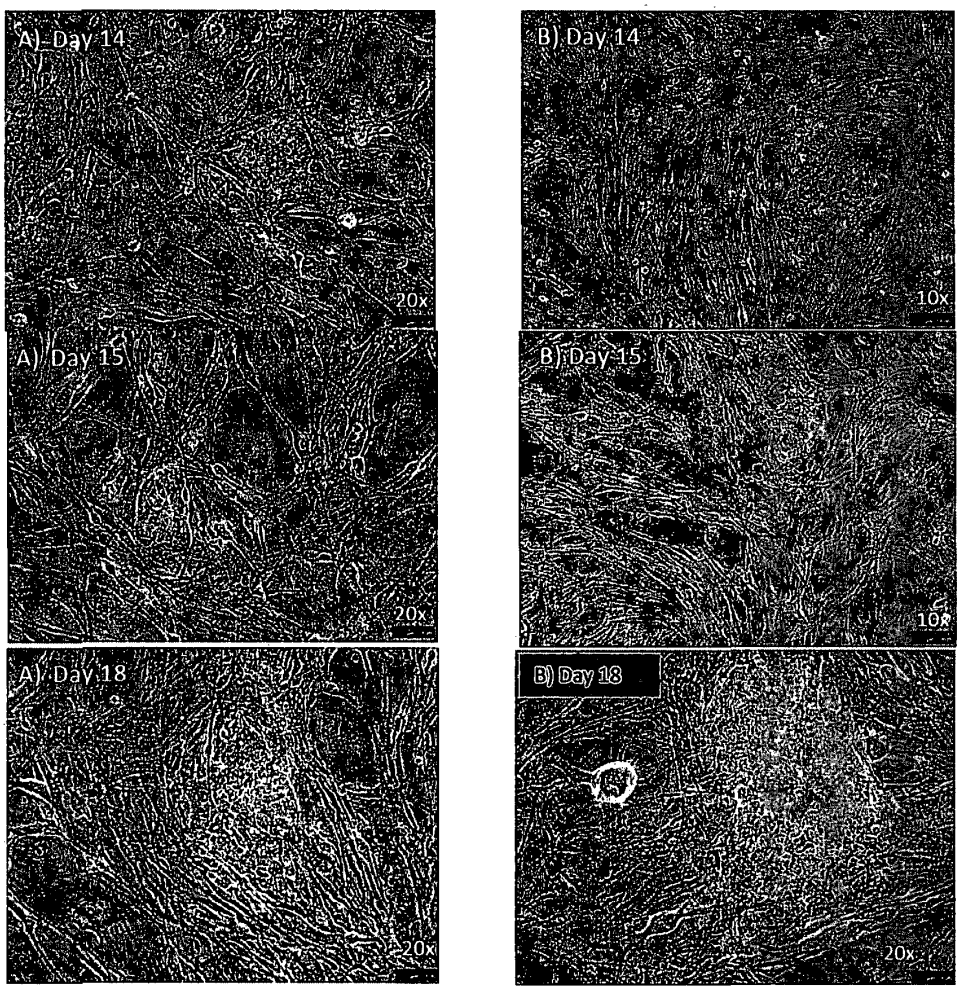

A) Day 14
20x
A) Day 15
20x
A) Day 18
20x
B) Day 14
10x
B) Day 15
10x
B) Day 18
20x

Fig. 3

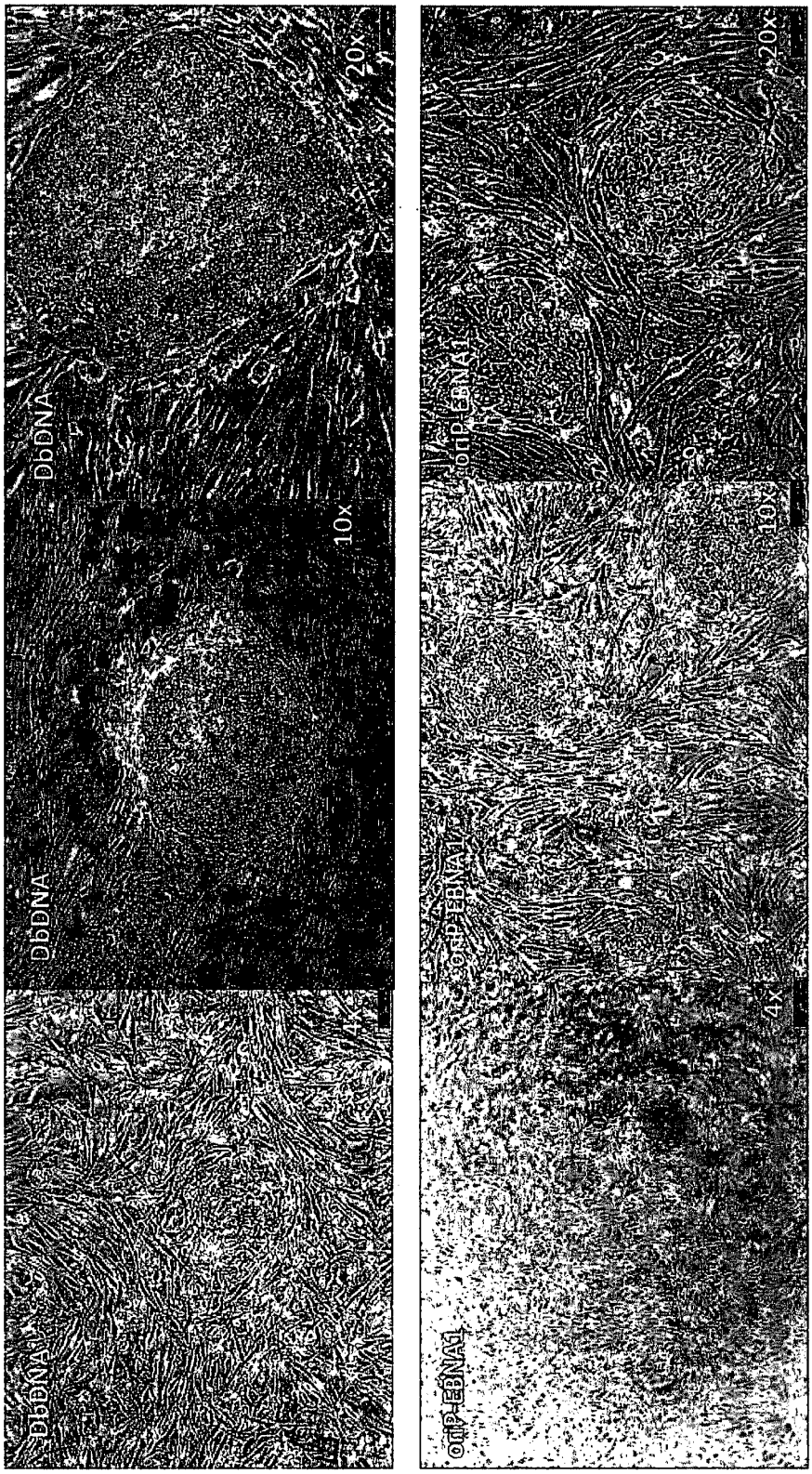

DbDNA
4x
DbDNA
10x
DbDNA
20x
oriP-EBNA1
4x
oriP-EBNA1
10x
oriP-EBNA1
20x

Fig. 4



Fig. 5

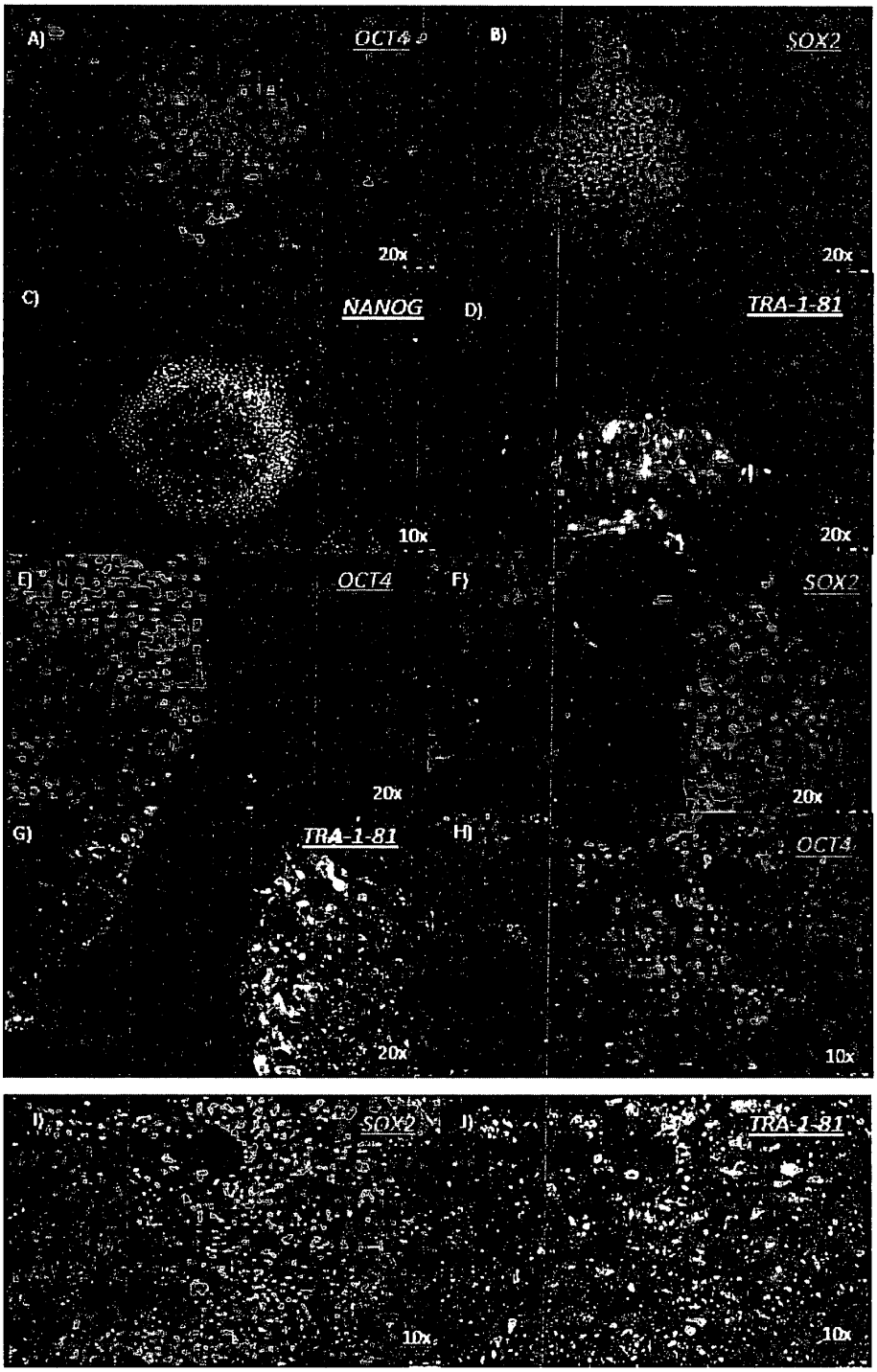

A)
OCT4
20x
B)
SOX2
20x
C)
NANOG
10x
D)
TRA-1-81
20x
E)
OCT4
20x
F)
SOX2
20x
G)
TRA-1-81
20x
H)
OCT4
10x
I)
SOX2
10x
J)
TRA-1-81
10x

Fig. 6

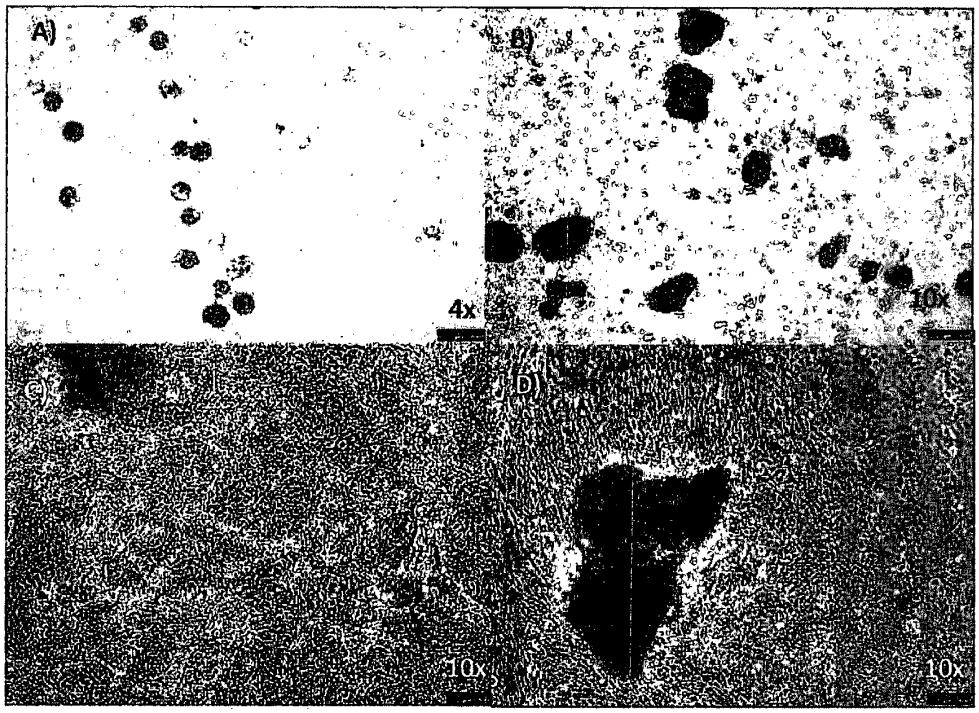


Fig. 7

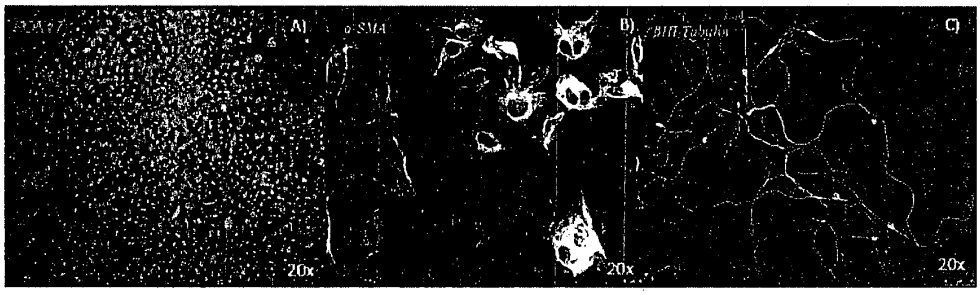


Fig. 8

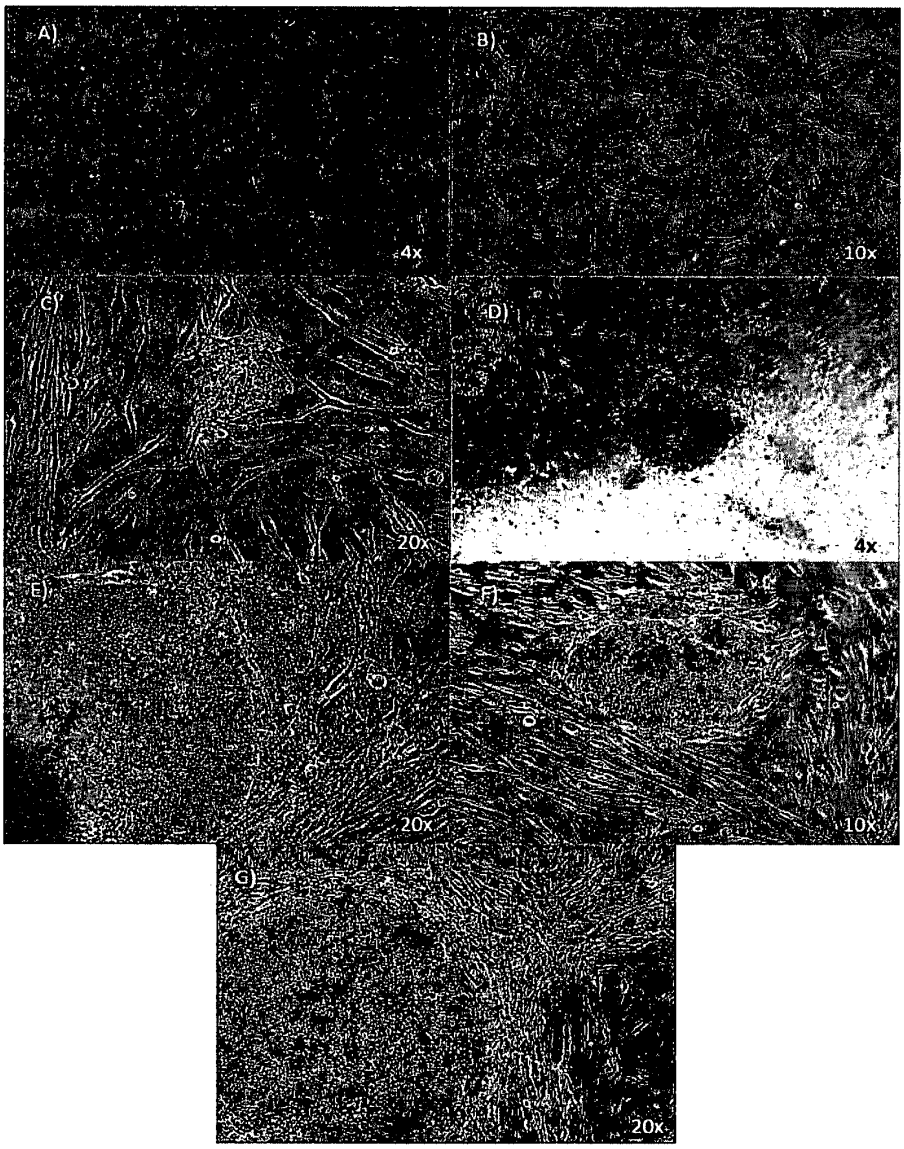
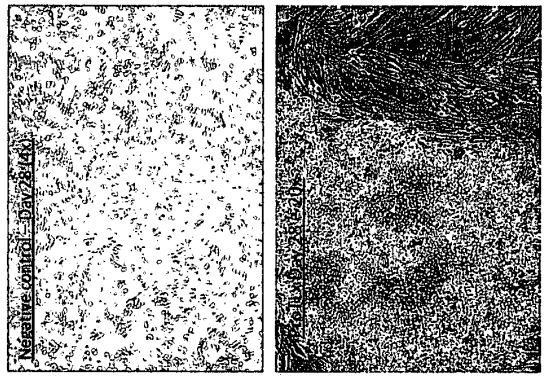


Fig. 9


Negative control – Day 28 (4x)
AP stain – negative

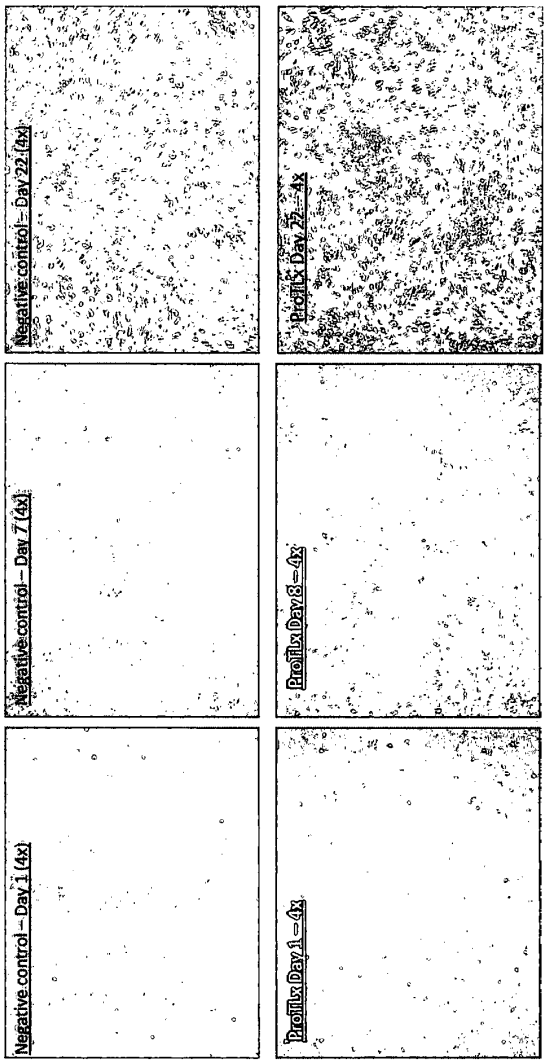

Negative control – Day 22 (4x)
Negative control – Day 7 (4x)
Negative control – Day 1 (4x)
Prolifx Day 25 – 4x
Prolifx Day 8 – 4x
Prolifx Day 1 – 4x

Fig. 10A

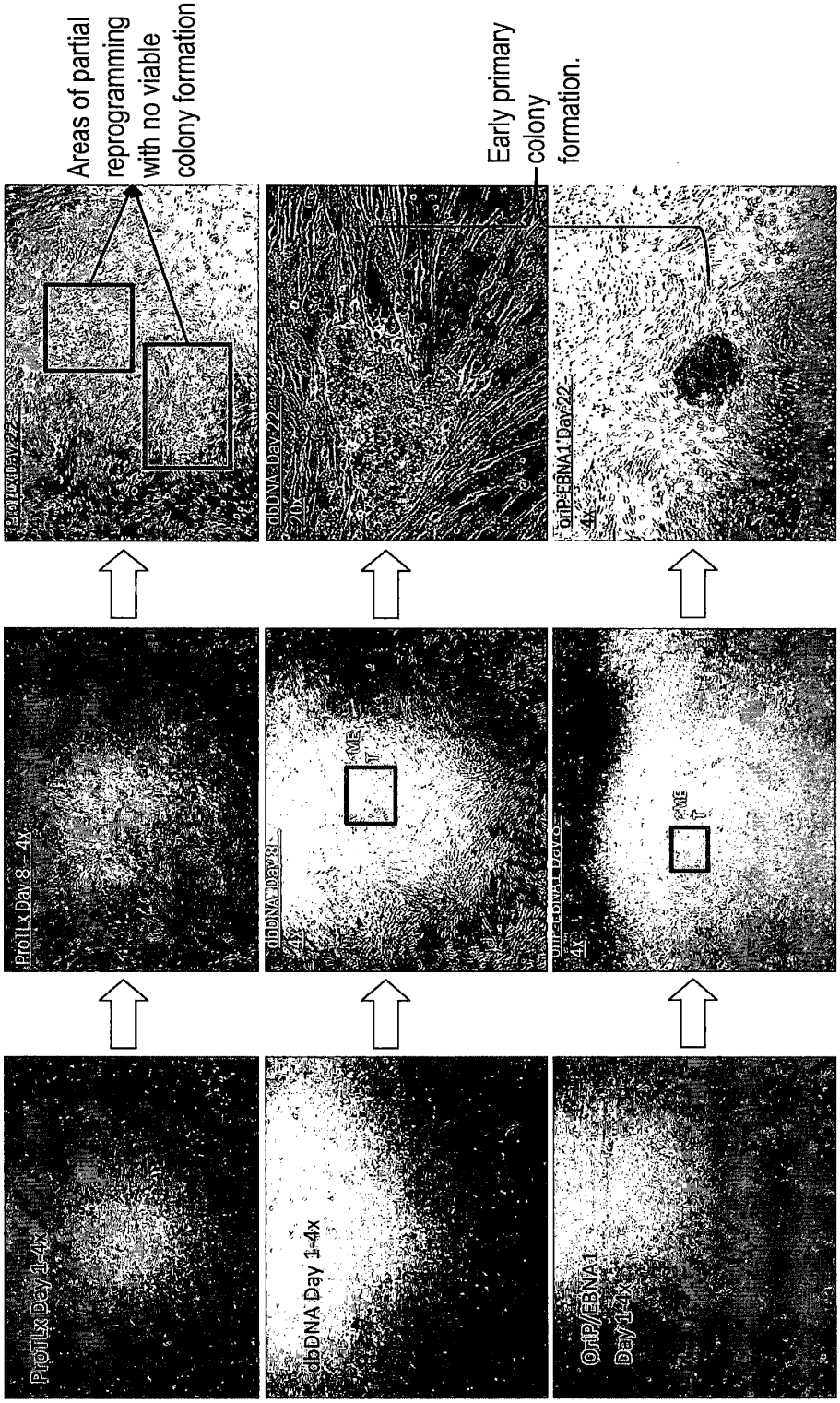

Areas of partial reprogramming with no viable colony formation
Early primary colony formation.
ProTLx Day 1-4x
dbDNA Day 1-4x
OriP/EBNA1 Day 1-4x
ProTLx Day 8 - 4x
dbDNA Day 8 - 4x
OriP/EBNA1 Day 8 - 4x
ProTLx Day 22 - 4x
dbDNA Day 22 - 20x
oriP/EBNA1 Day 22 - 4x

Fig. 10B

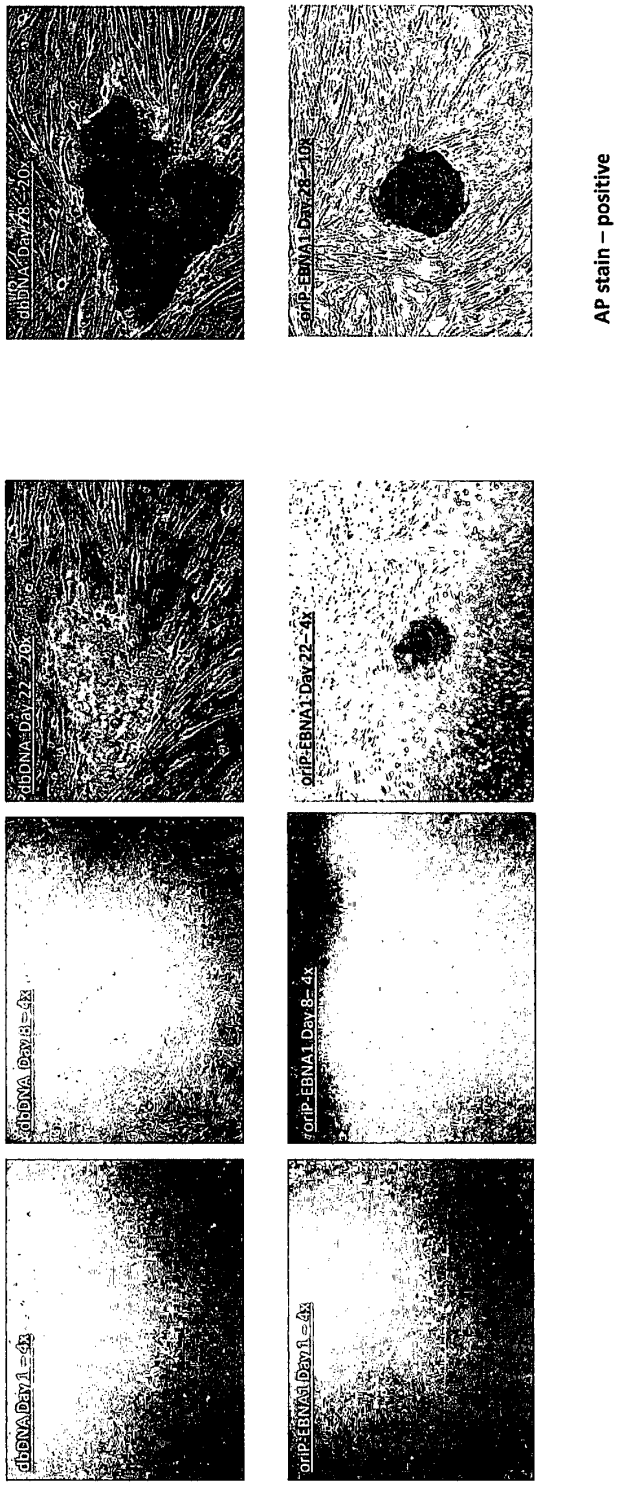

dbDNA Day 1 – 4x
dbDNA Day 8 – 4x
dbDNA Day 22 – 10x
dbDNA Day 28 – 10x
oriP-EBNA1 Day 1 – 4x
oriP-EBNA1 Day 8 – 4x
oriP-EBNA1 Day 22 – 4x
oriP-EBNA1 Day 28 – 10x
AP stain – positive

Fig. 11

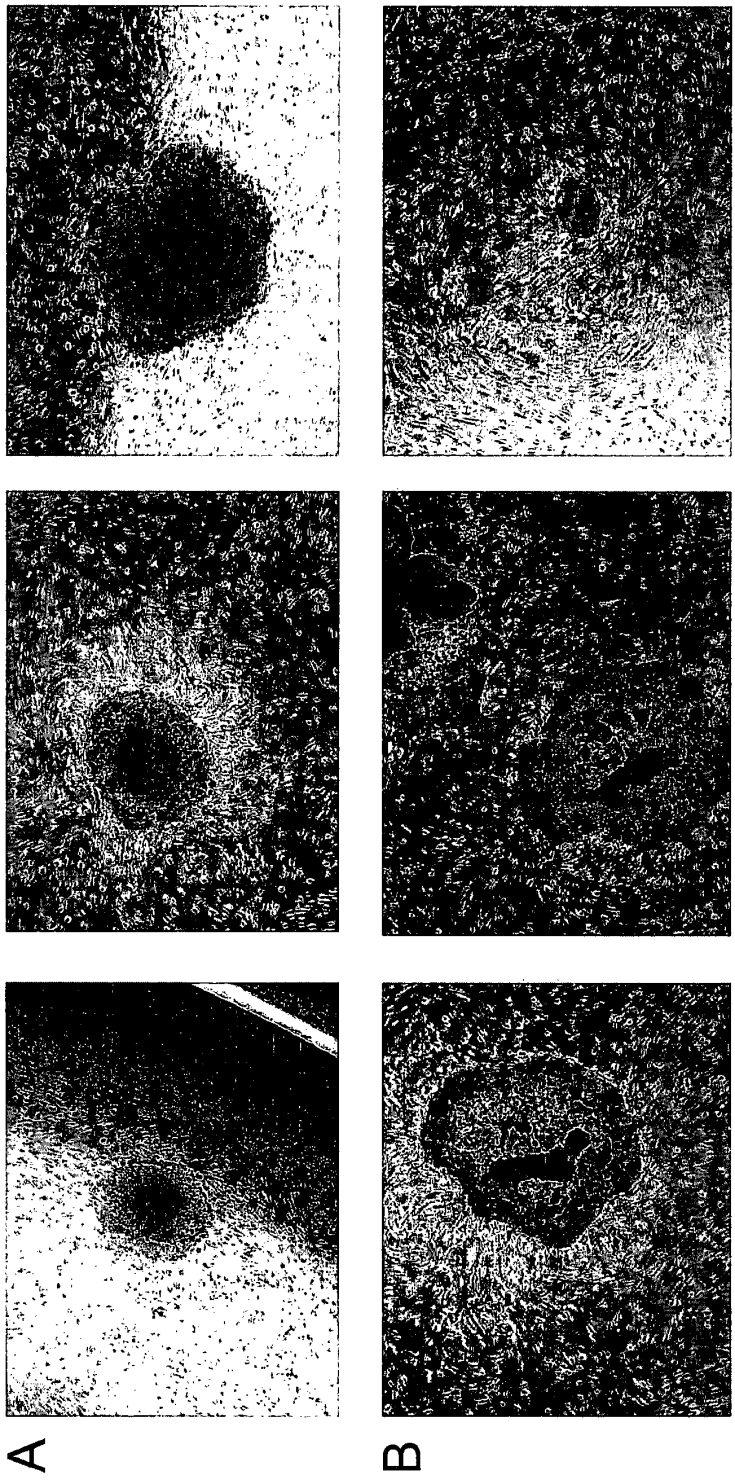


Fig. 12

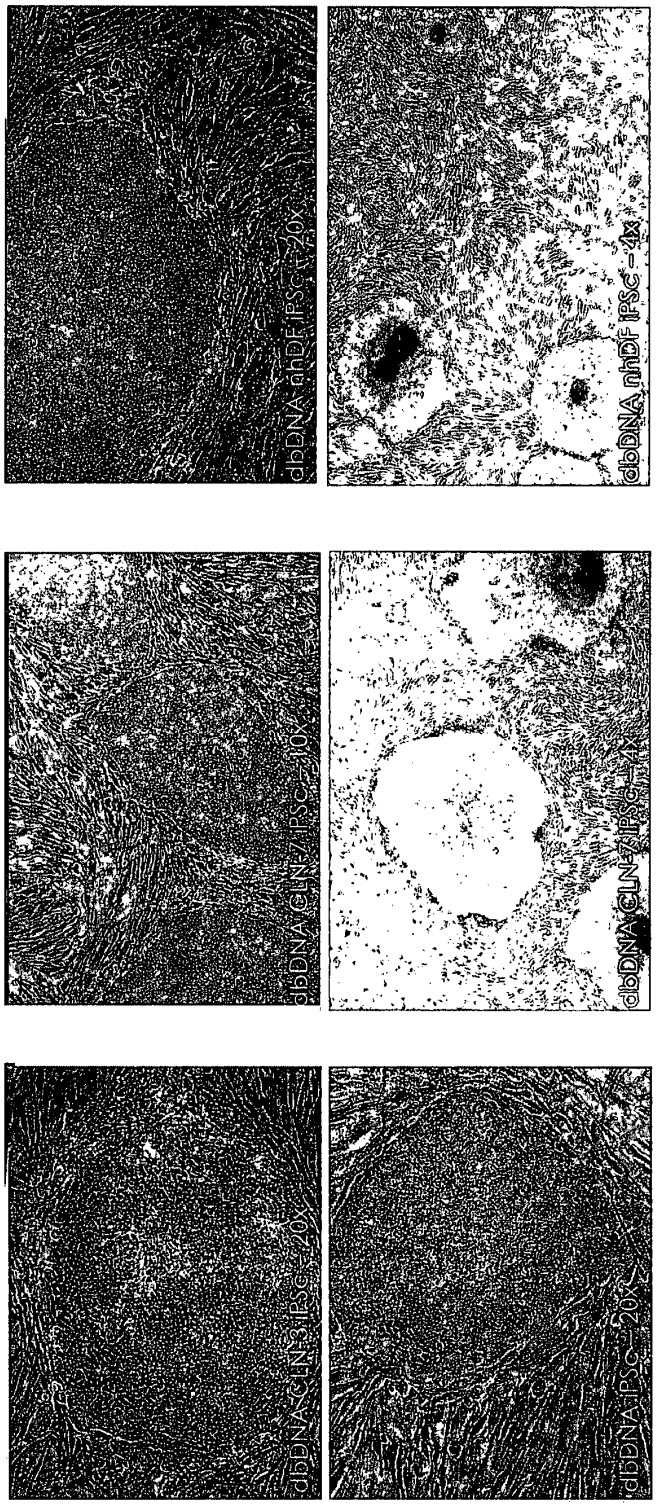

dbDNA nhDF iPSC - 20x
dbDNA nhDF iPSC - 4x
dbDNA CLN7 iPSC - 10x
dbDNA CLN7 iPSC - 4x
dbDNA CLN3 iPSC - 10x
dbDNA iPSC - 20x

Fig. 13

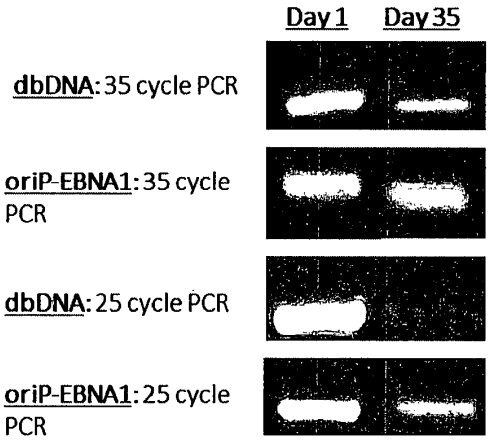


Fig. 14A

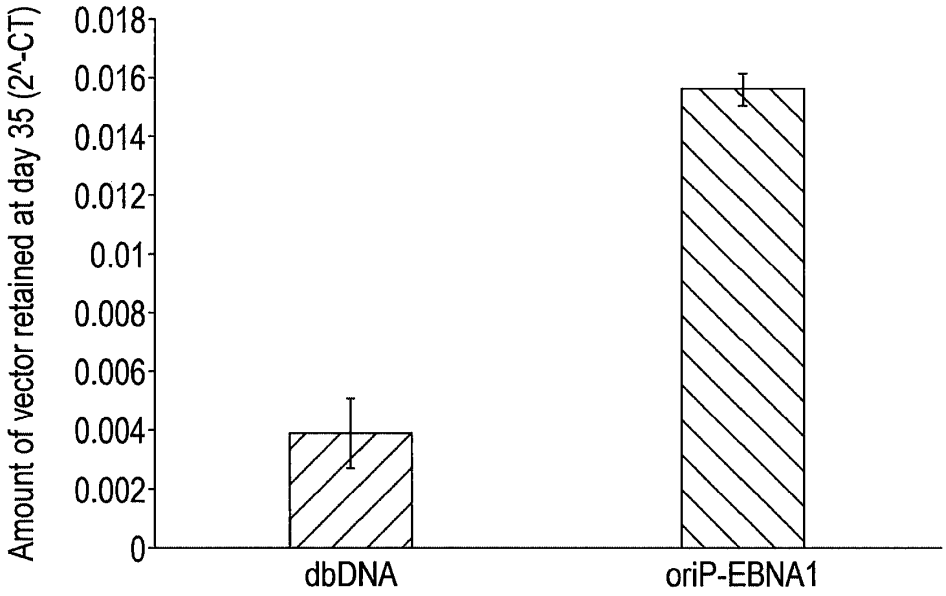


Fig. 14B

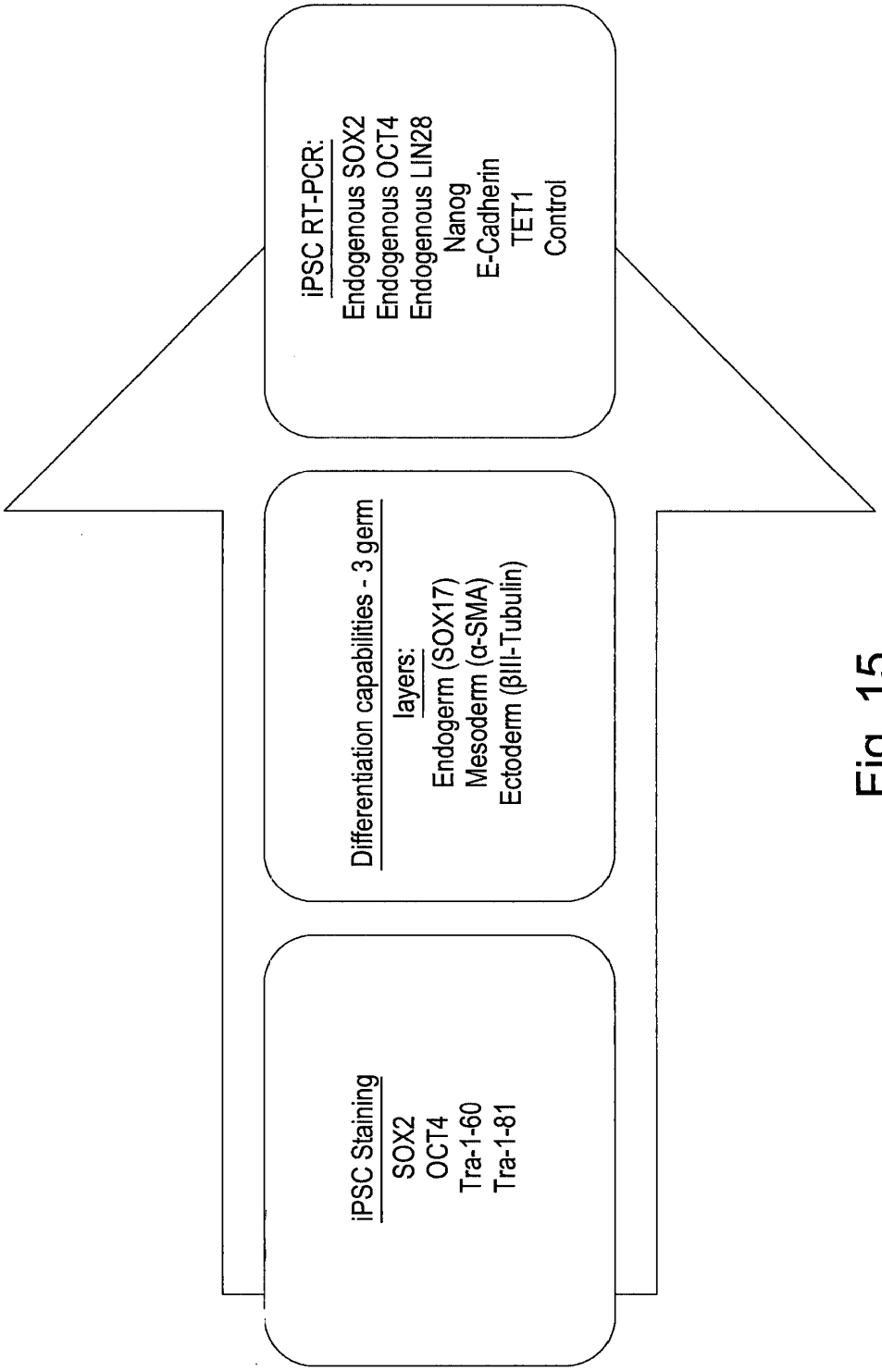

iPSC Staining
SOX2
OCT4
Tra-1-60
Tra-1-81
Differentiation capabilities - 3 germ layers:
Endogerm (SOX17)
Mesoderm (α-SMA)
Ectoderm (βIII-Tubulin)
iPSC RT-PCR:
Endogenous SOX2
Endogenous OCT4
Endogenous LIN28
Nanog
E-Cadherin
TET1
Control

Fig. 15

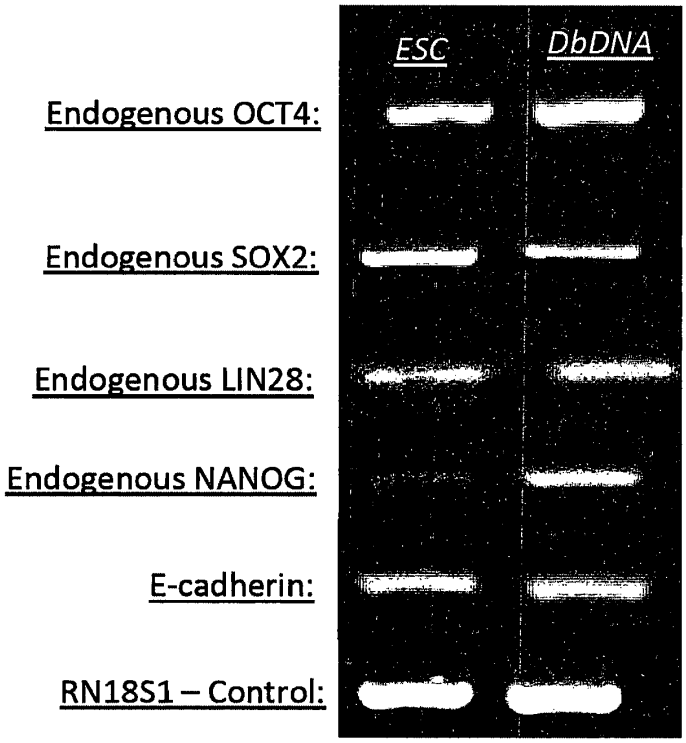

ESC
DbDNA
Endogenous OCT4:
Endogenous SOX2:
Endogenous LIN28:
Endogenous NANOG:
E-cadherin:
RN18S1 – Control:

Fig. 16

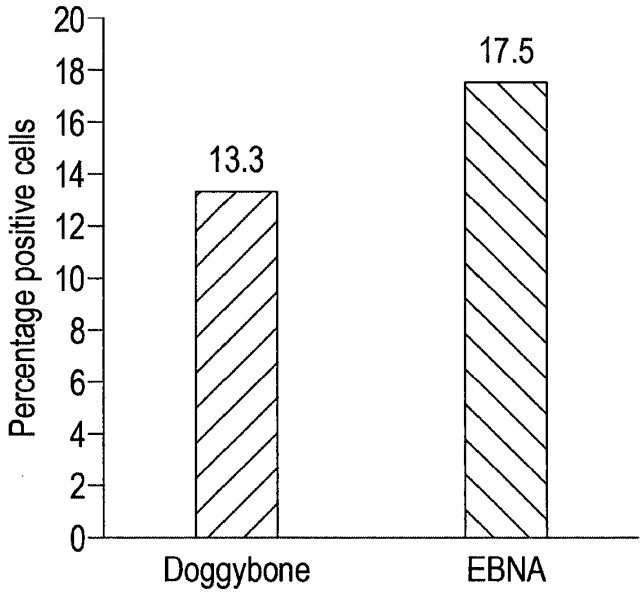

Percentage positive cells
20
18
16
14
12
10
8
6
4
2
0
13.3
17.5
Doggybone
EBNA

Fig. 17

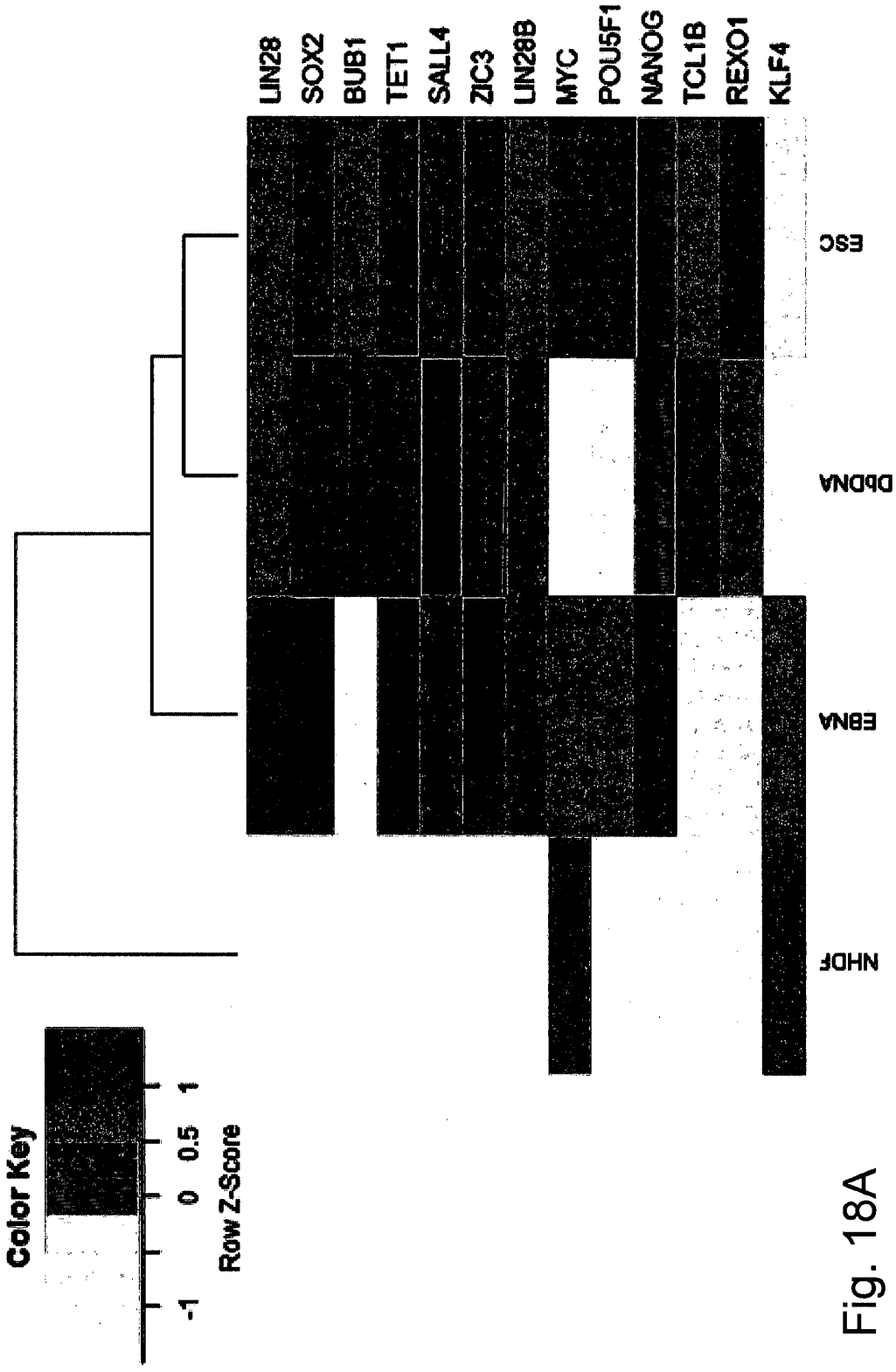

LIN28
SOX2
BUB1
TET1
SALL4
ZIC3
LIN28B
MYC
POU5F1
NANOG
TCL1B
REXO1
KLF4
ESC
DEDNA
EBNA
NHDF
Color Key
-1
0
0.5
1
Row Z-Score

Fig. 18A

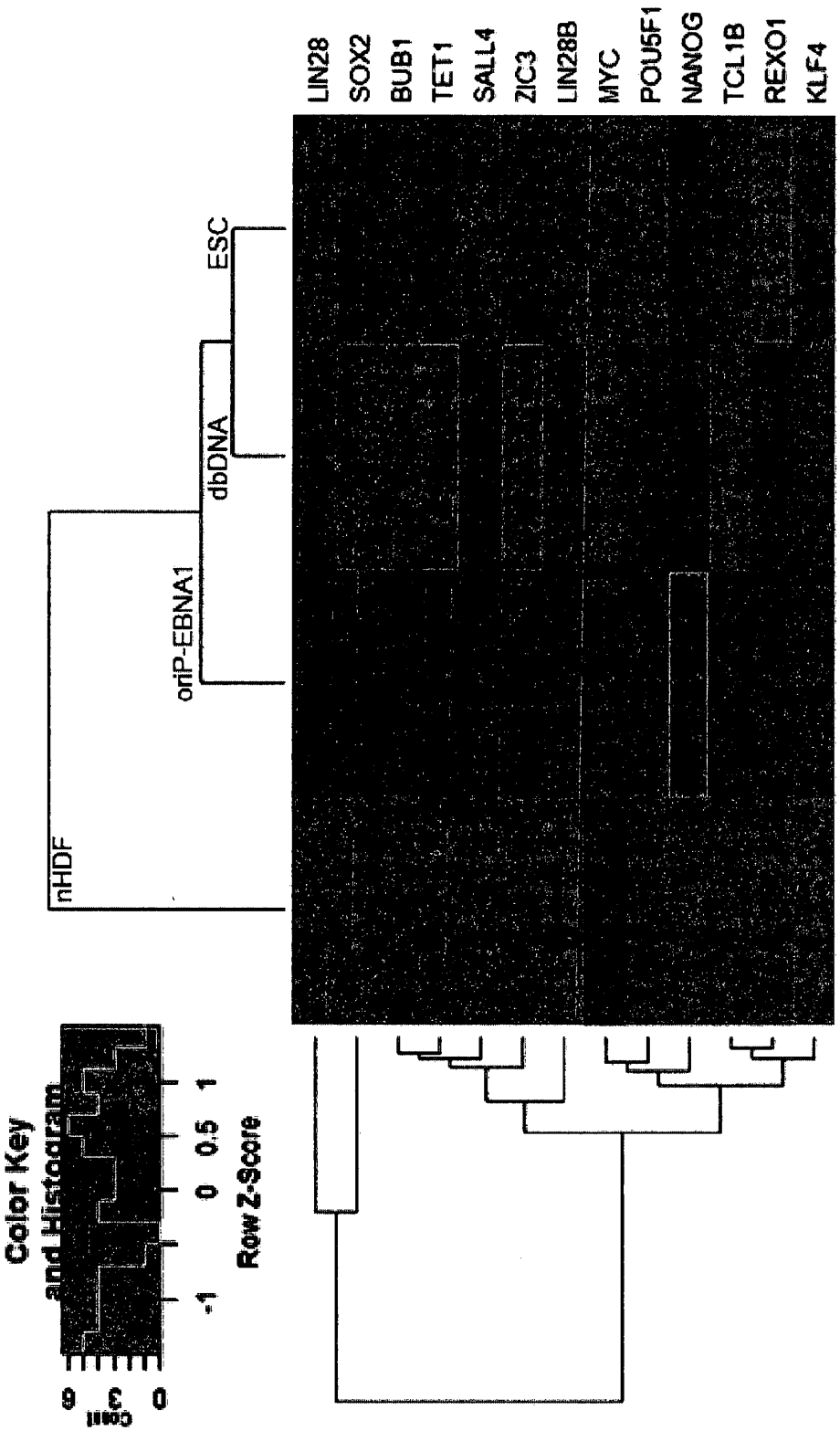

LIN28
SOX2
BUB1
TET1
SALL4
ZIC3
LIN28B
MYC
POU5F1
NANOG
TCL1B
REXO1
KLF4
ESC
dbDNA
oriP-EBNA1
nHDF
Color Key and Histogram
Count
0 3 6
-1 0 0.5 1
Row Z-Score

Fig. 18B

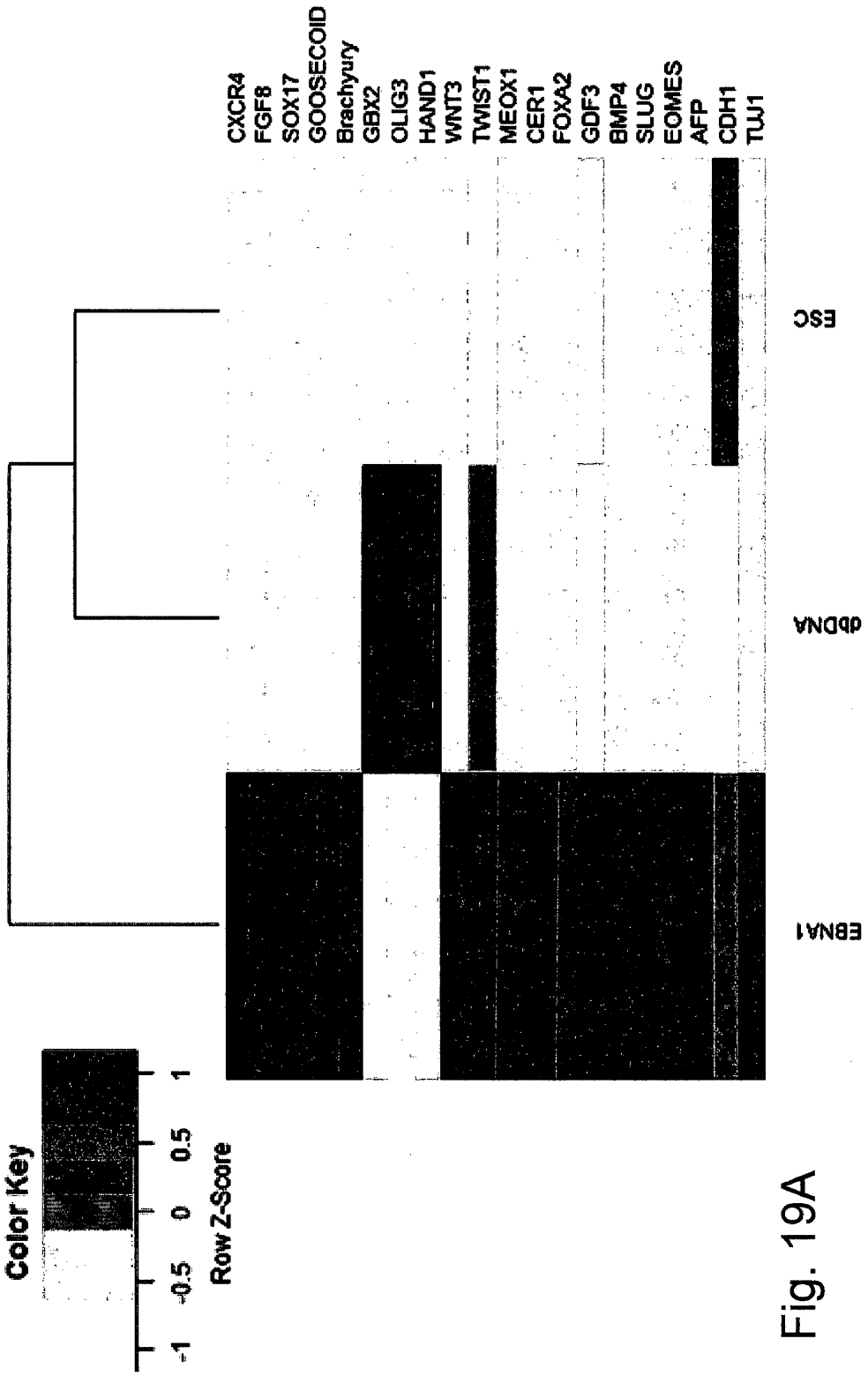

CXCR4
FGF8
SOX17
GOOSECOID
Brachyury
GBX2
OLIG3
HAND1
WNT3
TWIST1
MEOX1
CER1
FOXA2
GDF3
BMP4
SLUG
EOMES
AFP
CDH1
TUJ1
ESC
ddDNA
EBNA1
Color Key
-1
-0.5
0
0.5
1
Row Z-Score

Fig. 19A

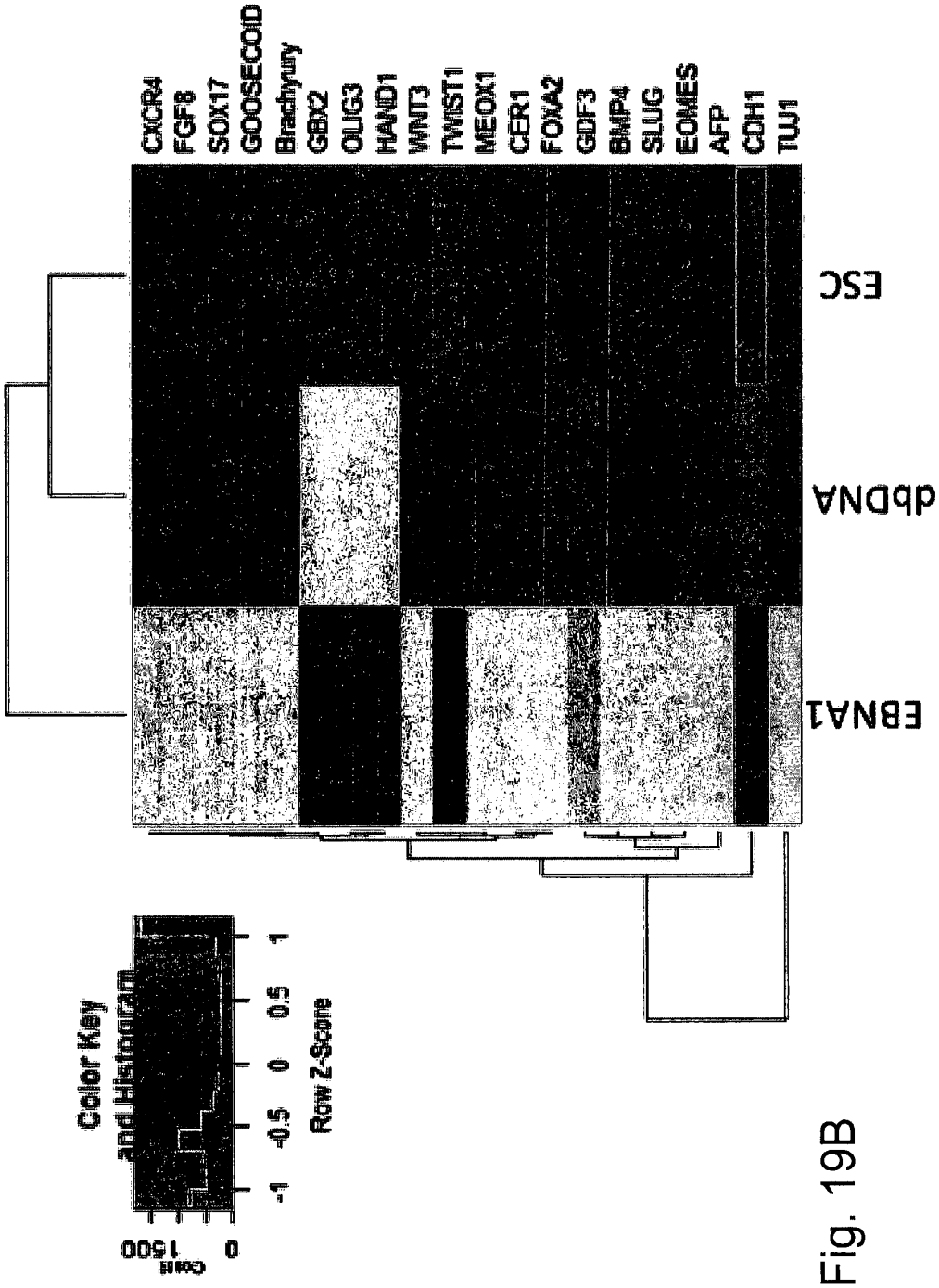

CXCR4
FGF8
SOX17
GOOSECOID
Brachyury
GBX2
OLIG3
HAND1
WNT3
TWIST1
MEOX1
CER1
FOXA2
GDF3
BMP4
SLUG
EOMES
AFP
CDH1
TUJ1
ESC
dbDNA
EBNA1
Color Key and Histogram
Row Z-Score
-1
-0.5
0
0.5
1
Count
0
1500

Fig. 19B

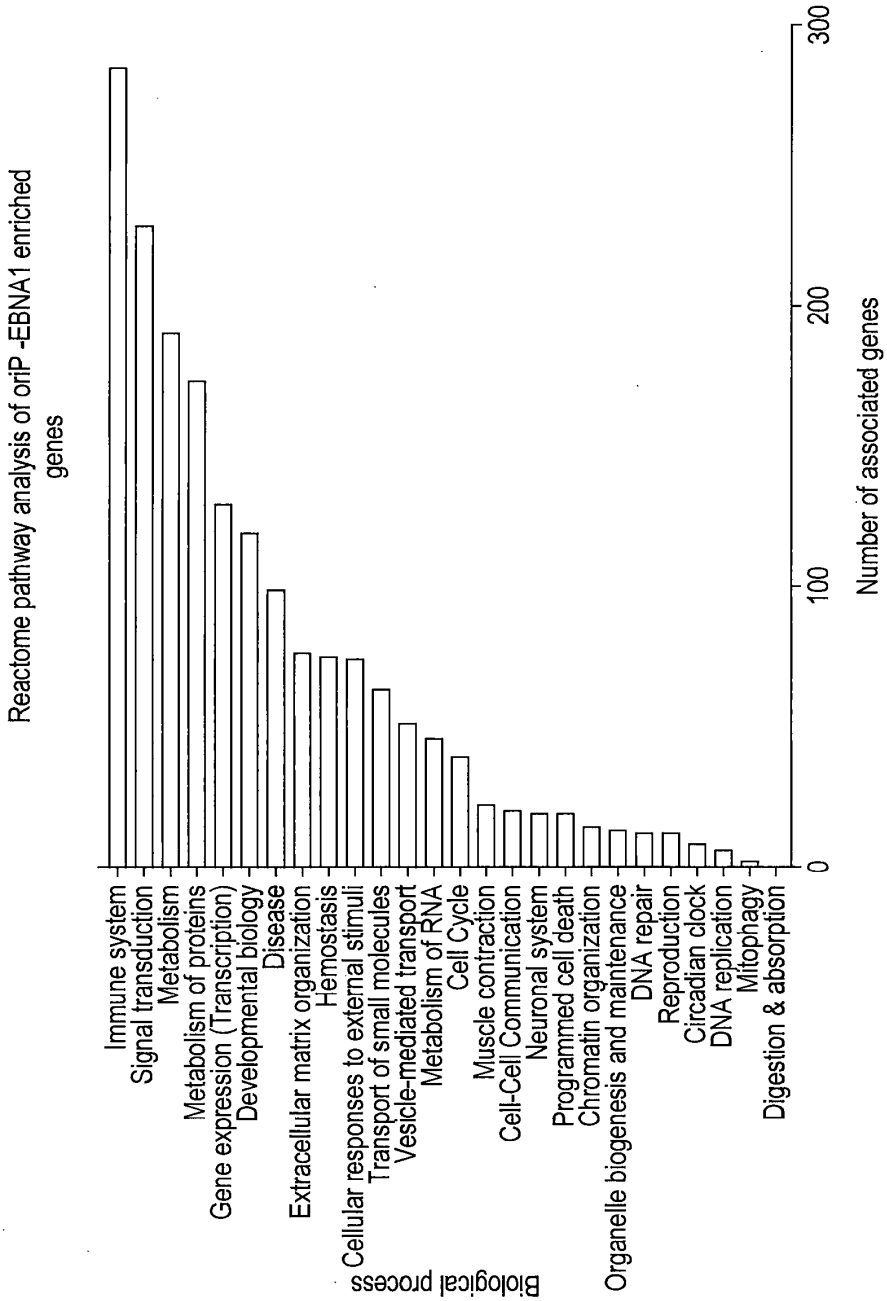

Reactome pathway analysis of oriP -EBNA1 enriched genes
Immune system
Signal transduction
Metabolism
Metabolism of proteins
Gene expression (Transcription)
Developmental biology
Disease
Extracellular matrix organization
Hemostasis
Cellular responses to external stimuli
Transport of small molecules
Vesicle-mediated transport
Metabolism of RNA
Cell Cycle
Muscle contraction
Cell-Cell Communication
Neuronal system
Programmed cell death
Chromatin organization
Organelle biogenesis and maintenance
DNA repair
Reproduction
Circadian clock
DNA replication
Mitophagy
Digestion & absorption
Biological process
0
100
200
300
Number of associated genes

Fig. 20A

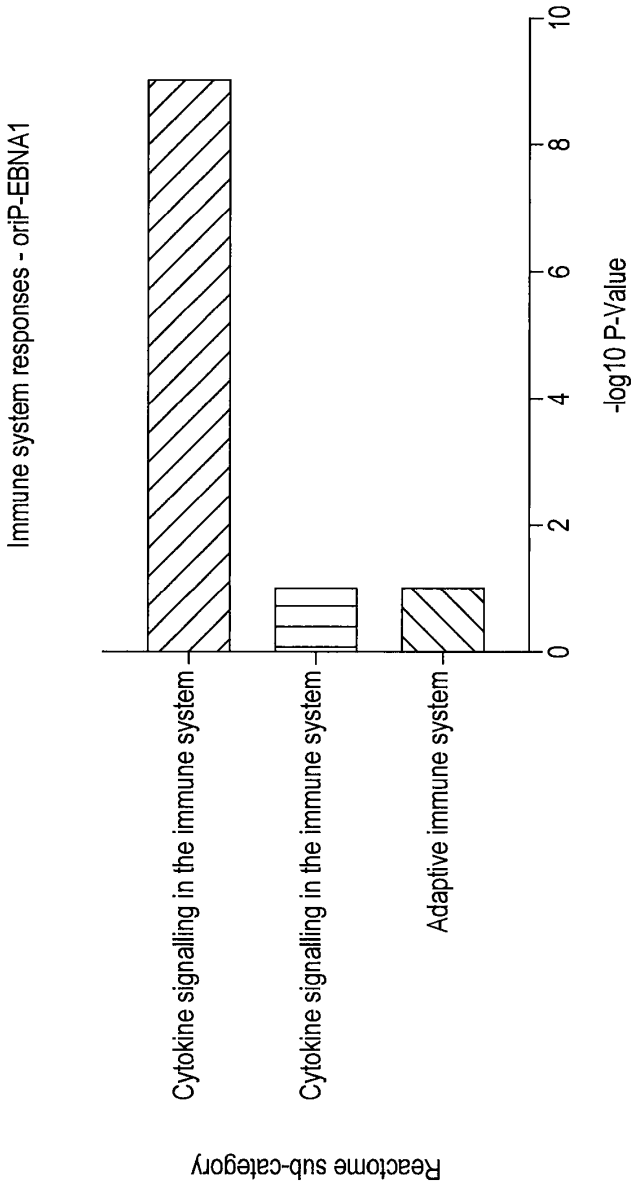

Immune system responses - oriP-EBNA1
0
2
4
6
8
10
-log10 P-Value
Cytokine signalling in the immune system
Cytokine signalling in the immune system
Adaptive immune system
Reactome sub-category

Fig. 20B

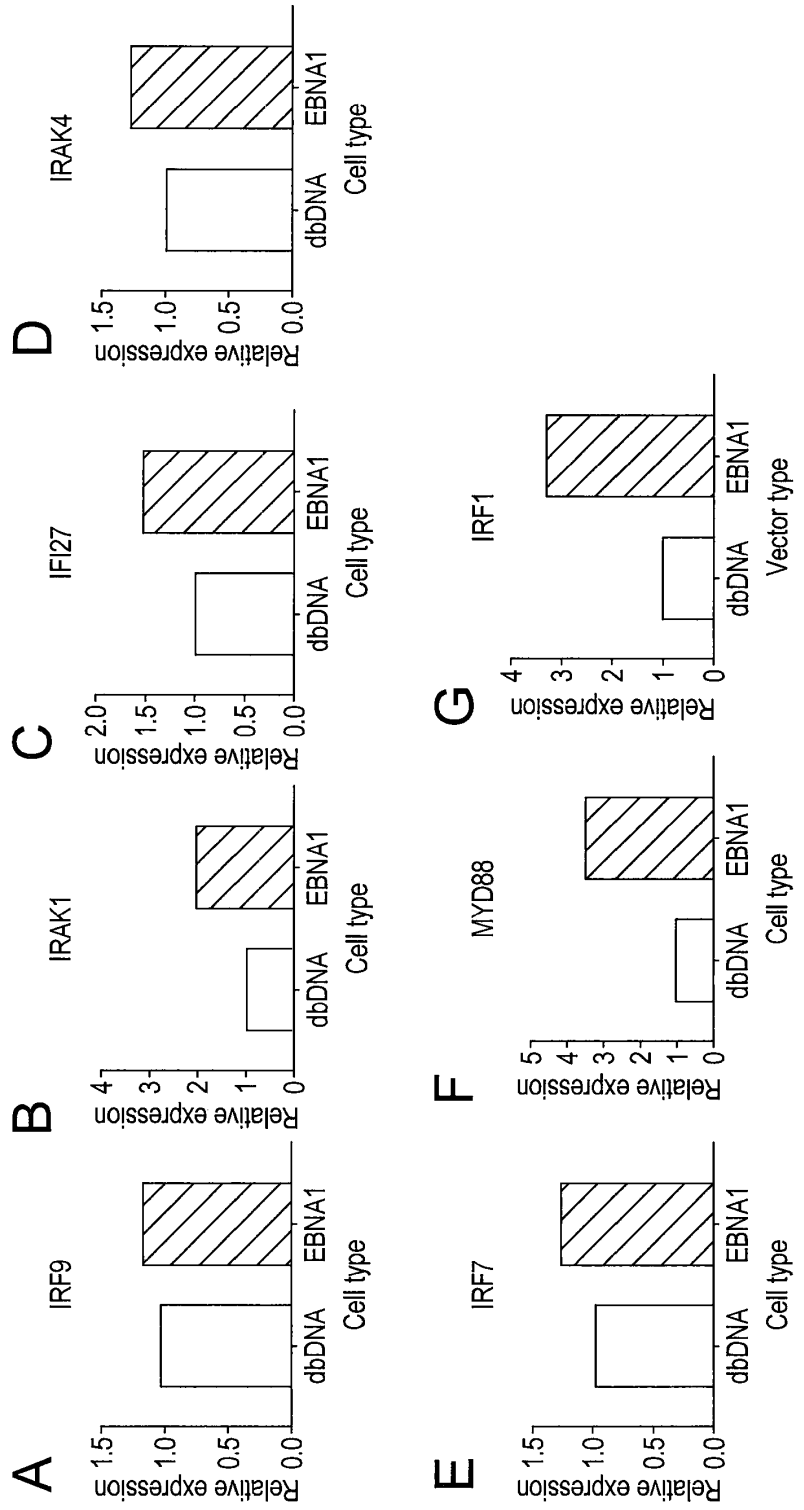

A
IRF9
Relative expression
1.5
1.0
0.5
0.0
dbDNA
EBNA1
Cell type
B
IRAK1
Relative expression
4
3
2
1
0
dbDNA
EBNA1
Cell type
C
IFI27
Relative expression
2.0
1.5
1.0
0.5
0.0
dbDNA
EBNA1
Cell type
D
IRAK4
Relative expression
1.5
1.0
0.5
0.0
dbDNA
EBNA1
Cell type
E
IRF7
Relative expression
1.5
1.0
0.5
0.0
dbDNA
EBNA1
Cell type
F
MYD88
Relative expression
5
4
3
2
1
0
dbDNA
EBNA1
Cell type
G
IRF1
Relative expression
4
3
2
1
0
dbDNA
EBNA1
Vector type

Fig. 21

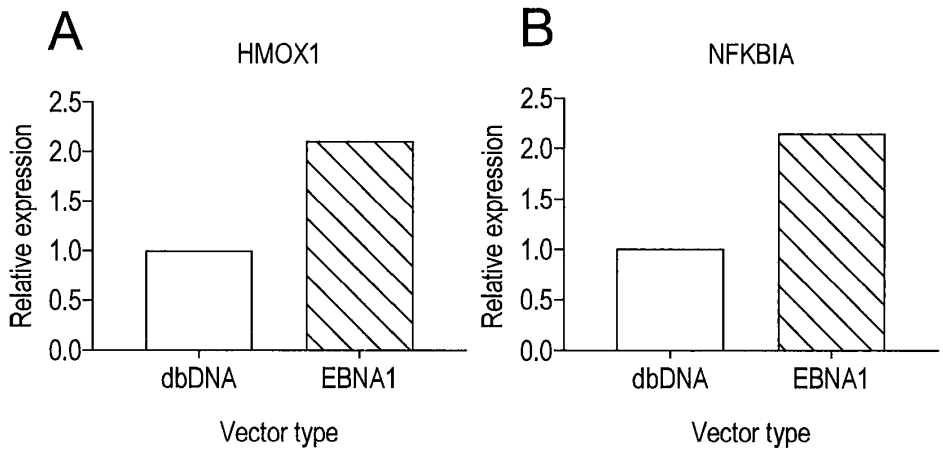

A
HMOX1
Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
dbDNA
EBNA1
Vector type
B
NFKBIA
Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
dbDNA
EBNA1
Vector type

Fig. 22

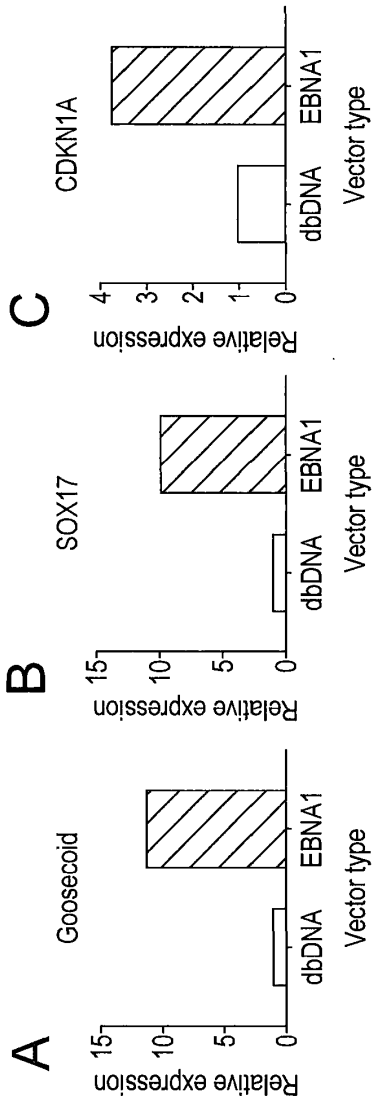

A
Goosecoid
Relative expression
0
5
10
15
dbDNA
EBNA1
Vector type
B
SOX17
Relative expression
0
5
10
15
dbDNA
EBNA1
Vector type
C
CDKN1A
Relative expression
0
1
2
3
4
dbDNA
EBNA1
Vector type

Fig. 24