# Figure 1

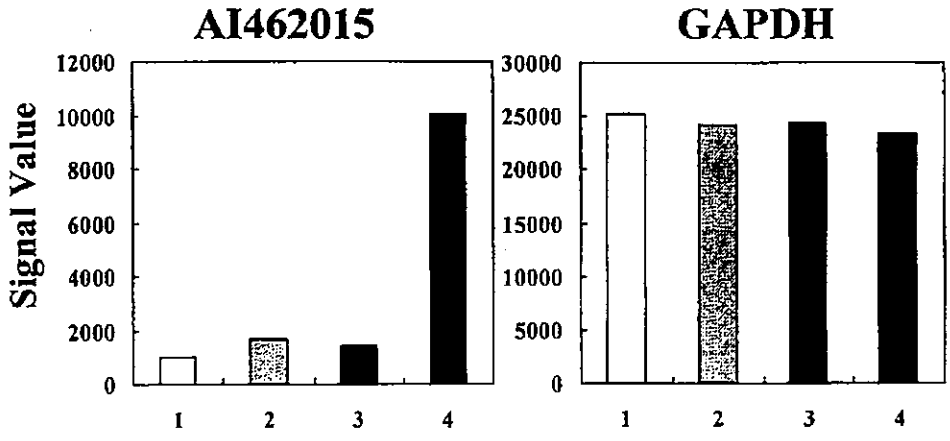



AAGTACCAAAATAATTACCAAC **AI462015**
**(437 bp)**

TTTGTCTGTAAAAATCTGTTTAATAAATATACATCTTAGAAGTACCAAAA
TAATTACCAACAAAATACAACATATACAACATTTACAAGAAGGCGACACA
GACCTTAGTTGGGGGCGACTTTTAAGCACATGCCACTGAACACCTGGCTC
TTACATGGGAGGACACACTGGGCTCACTTACTAGGTCTATGGTGGTTCAA
TCAAAAGCACAATAAATAAAACGTGGTCCTTTCATTAGGTTCTGGAAAAT
CACCTCCCCCCCCCCCAAAAAAAATCCCACAAACATGAACCTTAAGAGAC
ATTTTCTTTGAATTTCAGTGATCTGTTTCCCCGGATTTCACAAAGACAAC
AGCCGAATCACCCCAGTAAAATGCCTGGGTCTAGGCGCTGTGTGGTGTGG
TGCTAAGTATACCCTTTCTCATTTTTTTTCTTTTTCT

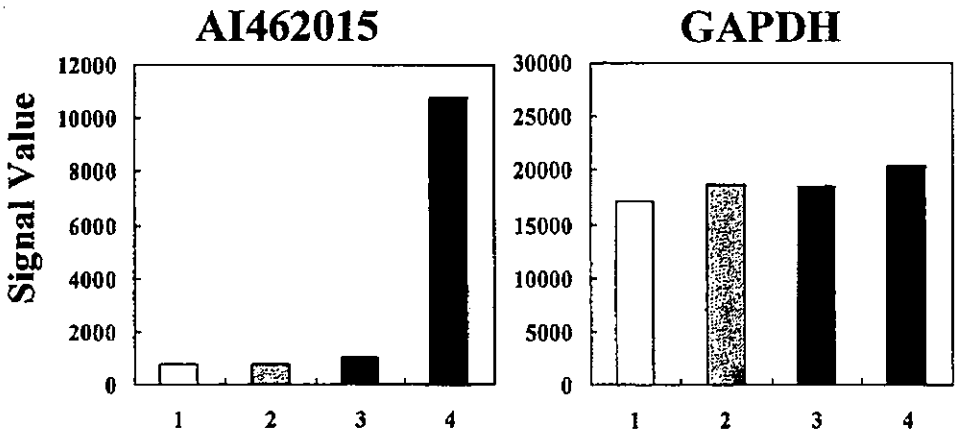
**LOCUS AI462015 437 bp mRNA linear EST 09-MAR-1999**
**DEFINITION ub69d10.x1 Soares_mammary_gland_NMLMG Mus musculus cDNA clone IMAGE:1382995 3' similar to gb:M69043 MAJOR HISTOCOMPATIBILITY COMPLEX ENHANCER-BINDING PROTEIN (HUMAN);, mRNA sequence.**
**ACCESSION AI462015**
**VERSION AI462015.1 GI:4316045**

# Figure 2



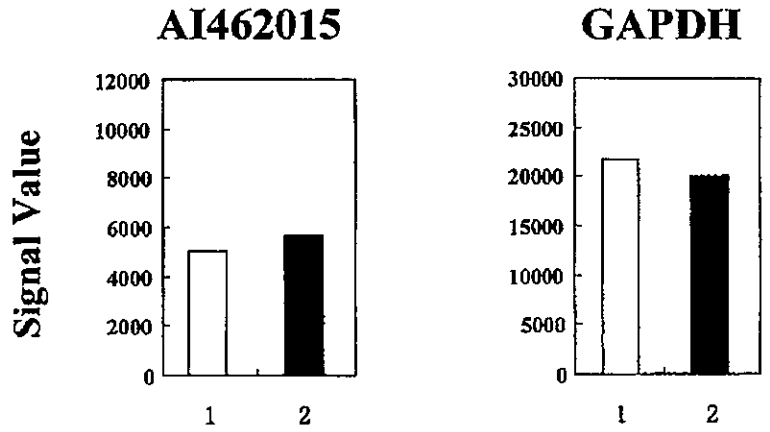

| | | | |
|---|---|---|---|
| 1- | | | DG44 cell |
| 2- | | TAUT-overexpressing | DG44 cell |
| 3- | | TAUT-overexpressing and CSAD-overexpressing | DG44 cell |
| 4- | Strongly MAb1 (anti-IL-6R antibody)-expressing | TAUT-overexpressing, and CSAD-overexpressing | DG44 cell |

# Figure 3




1- TAUT-overexpressing DXB11s cell
2- CSAD-overexpressing DXB11s cell
3- AE1-overexpressing DXB11s cell
4- Strongly MAb2 (anti-glypican 3 antibody)-expressing DXB11s cell

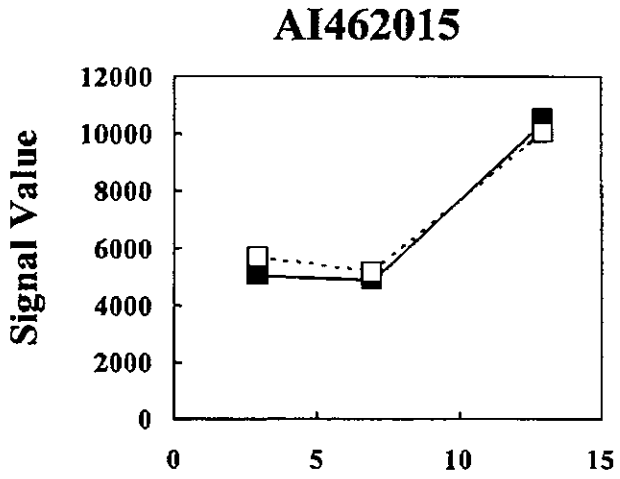
# Figure 4




1- Strongly MAb2 (anti-glypican 3 antibody)-expressing DXB11s cell

2- Strongly MAb2 (anti-glypican 3 antibody)-expressing and TAUT-overexpressing DXB11s cell

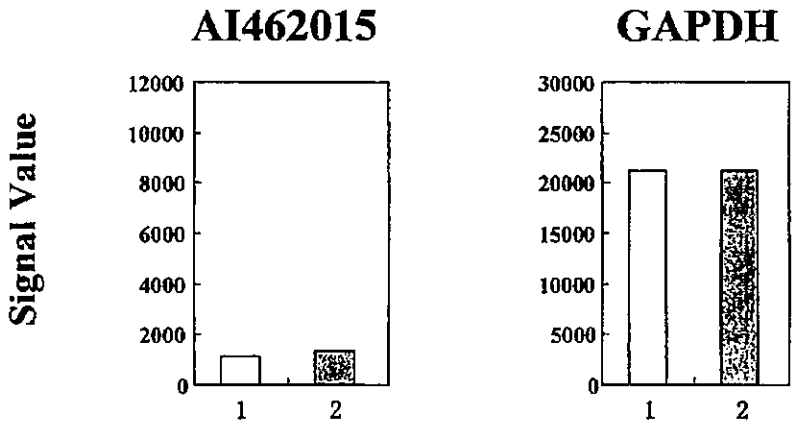
# Figure 5




■ 1- Strongly MAb2 (anti-glypican 3 antibody)-expressing DXB11s cell

□ 2- Strongly MAb2 (anti-glypican 3 antibody)-expressing and TAUT-overexpressing DXB11s cell

# Figure 6




□ 1- Strongly MAb1 (anti-IL-6R antibody)-expressing DXB11s cell (Hy-Fish addition)

□ 2- Strongly MAb1 (anti-IL-6R antibody)-expressing DXB11s cell (porcine lysate addition)

*Figure 7*

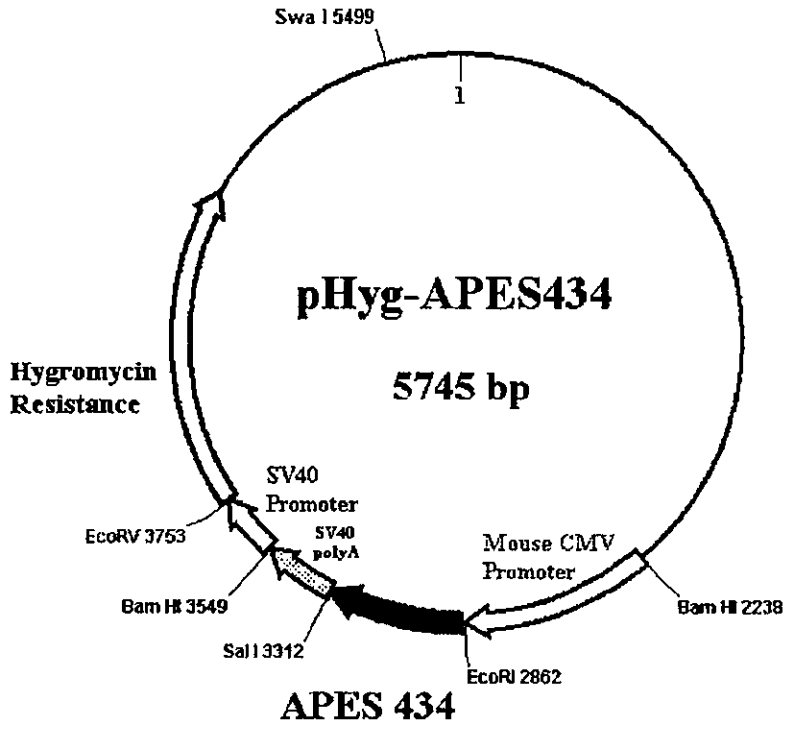



*Figure 8*

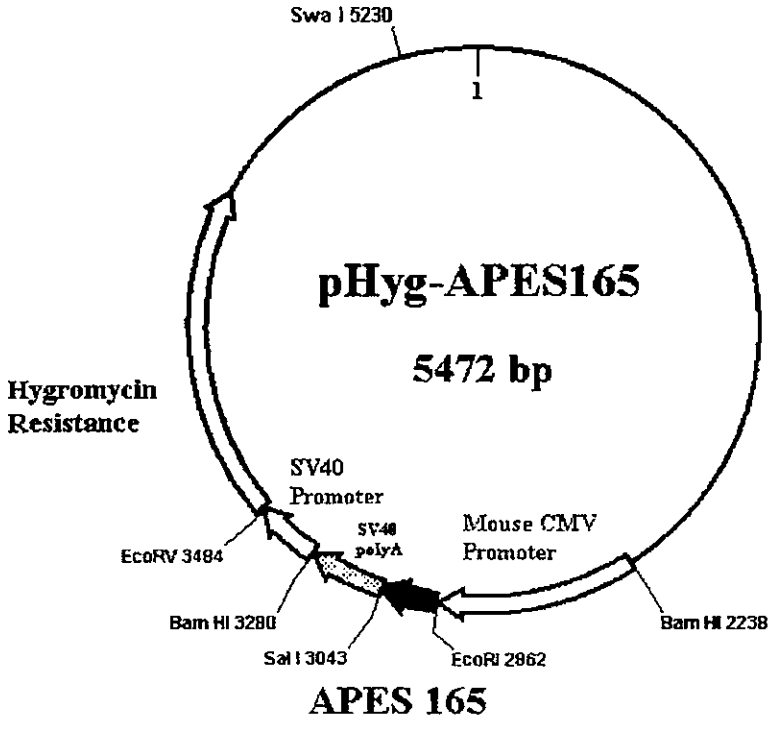


*Figure 9*

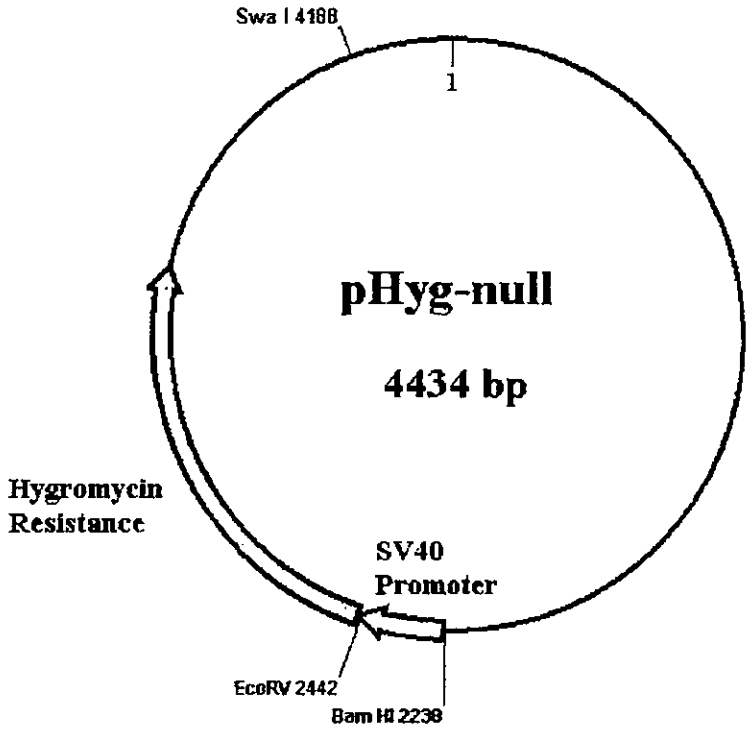
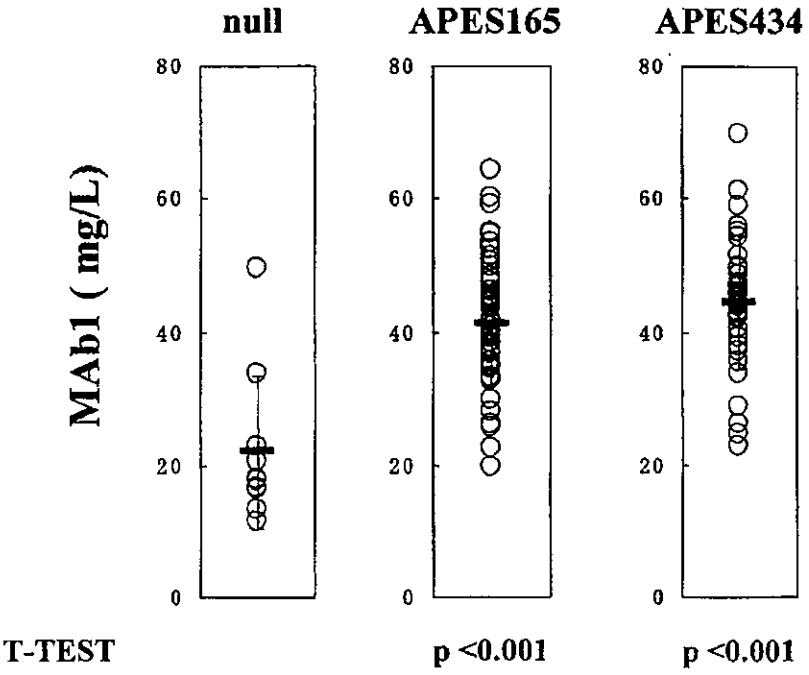
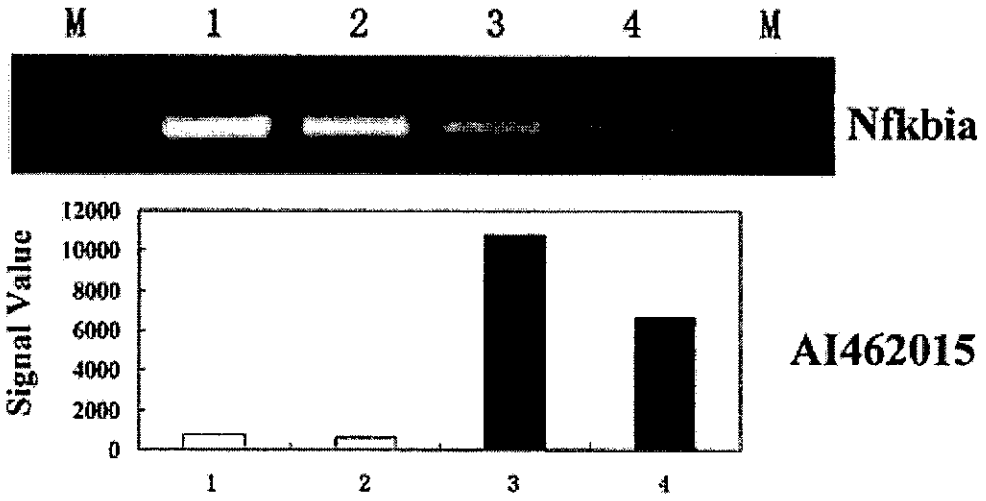



*Figure 10*



# Figure 11




M- DNA molecular weight Marker ( λ/HindIII + φX174/HaeIII )

1- TAUT-overexpressing DXB11s cell

2- CSAD-overexpressing DXB11s cell

3- Strongly MAb2 (anti-glypican 3 antibody)-expressing DXB11s cell

4- Strongly MAb2 (anti-glypican 3 antibody)-expressing and TAUT-overexpressing DXB11s cell

# Figure 12

**A part of Hamster Nfkbia mRNA (134 bp)**

```
          10         20         30         40         50
agtacccggatacagcagcagctgggccagctgacccgggaaaatcttca

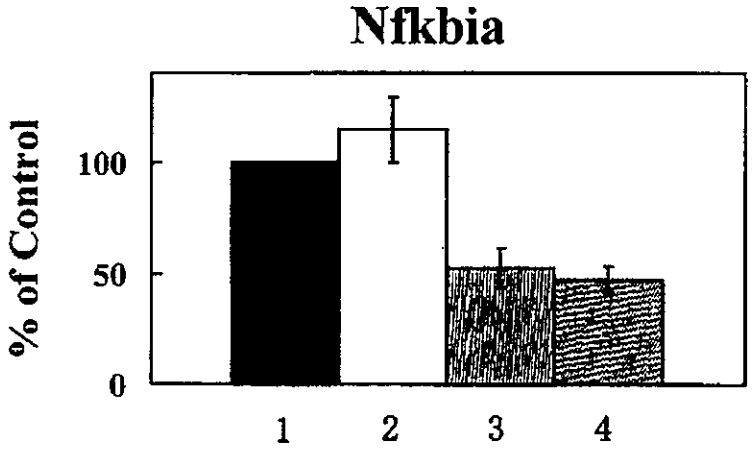
          60         70         80         90         100
gatgctgcccgagagtgaggatgaggagagctacgacacagagtcagaat

         110        120        130
tcacggaggatgagctgccctatgatgactgtgt
```

**TaqMan Probe Set for Hamster Nfkbia mRNA**

| | | | |
|---|---|---|---|
| 5′- cagctgacccgggaaaatc | Tm 59℃ | 19mer |
| 5′- tgactctgtgtcgtagctctcctc | Tm 59℃ | 24mer |
| 5′- FAM-tcagatgctgcccgagagtgagga-TAMRA | Tm 68℃ | 24mer |

# Figure 13



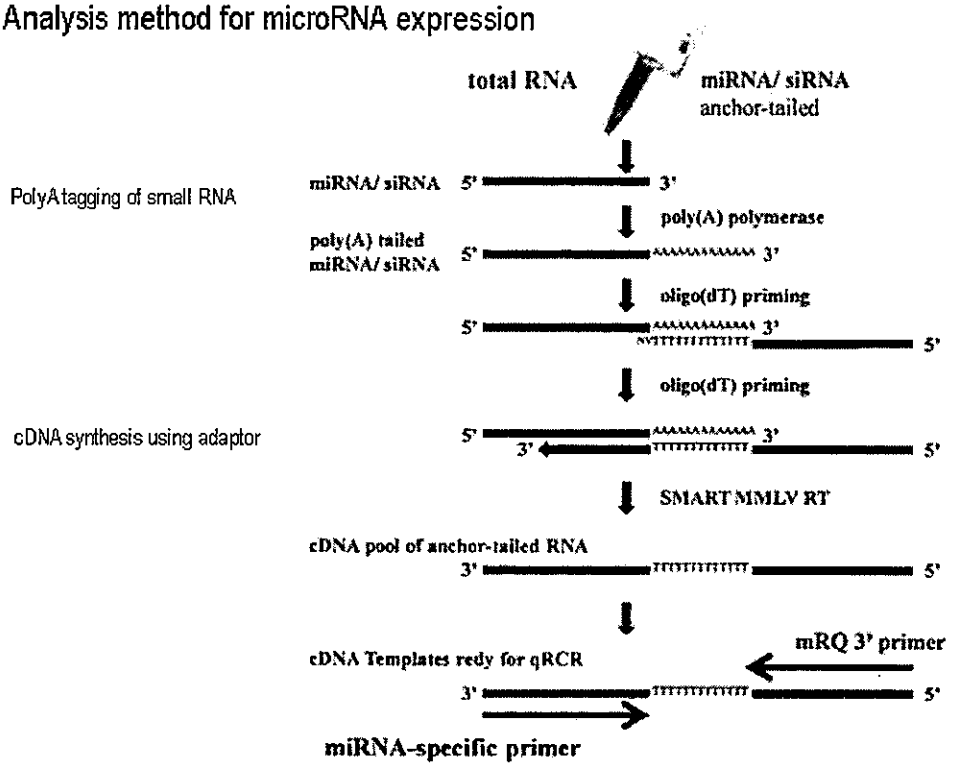

| | | |
|---|---|---|
| 1- | TAUT-overexpressing | DXB11s cell |
| 2- | CSAD-overexpressing | DXB11s cell |
| 3- Strongly MAb2 (anti-glypican 3 antibody)-expressing | | DXB11s cell |
| 4- Strongly MAb2 (anti-glypican 3 antibody)-expressing and TAUT-overexpressing | | DXB11s cell |

# Figure 14

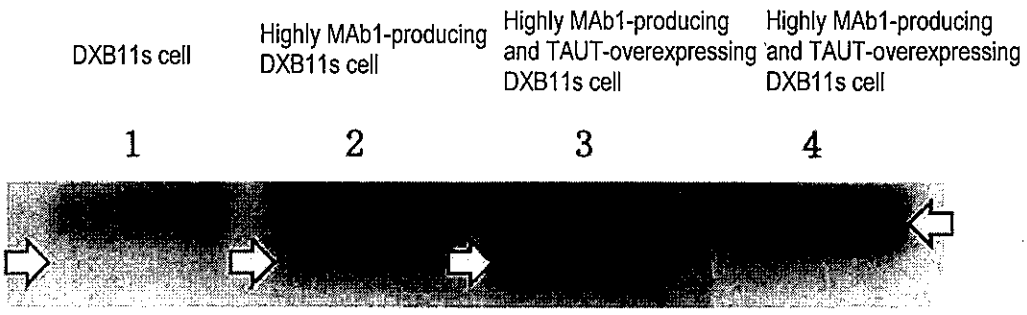
ctctgggctcgaatggcatgggggacagcttttatatggttaactccgcccgttttatgactagaaccaatagttttaatgccaaa
tgcactgaaatcccctaatttgcaaagccaaacgccccctatgtgagtaatacggggacttttacccaatttcccaagcggaaa
gccccctaatacactcatatggcatatgaatcagcacggtcatgcactctaatggcggcccatagggactttccacataggggg
gcgttcaccatttcccagcataggggtggtgactcaatggcctttacccaagtacattgggtcaatgggaggtaagccaatgg
gtttttcccattactggcaagcacactgagtcaaatgggactttccactgggttttgcccaagtacattgggtcaatgggaggtga
gccaatgggaaaaacccattgctgccaagtacactgactcaatagggactttccaatgggtttttccattgttggcaagcatata
aggtcaatgtgggtgagtcaatagggactttccattgtattctgcccagtacataaggtcaatagggggtgaatcaacaggaaa
gtcccattggagccaagtacactgcgtcaatagggactttccattgggttttgcccagtacataaggtcaatagggggatgagtc
aatgggaaaaacccattggagccaagtacactgactcaatagggactttccattgggttttgcccagtacataggtcaatagg
gggtgagtcaacaggaaagttccattggagccaagtacattgagtcaatagggactttccaatgggttttgcccagtacataag
gtcaatgggaggtaagccaatgggttttcccattactggcacgtatactgagtcattagggactttccaatgggttttgcccagt
acataaggtcaataggggtgaatcaacaggaaagtcccattggagccaagtacactgagtcaatagggactttccattgggttt
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attgggtttttccagccaatttaattaaaacgccatgtactttcccaccattgacgtcaatgggctattgaaactaatgcaacgtga
cctttaaacggtactttcccatagctgattaatgggaaagtaccgttctcgagccaatacacgtcaatgggaagtgaaagggca
gccaaaacgtaacaccgccccggttttccccctggaaattccatattggcacgcattctattggctgagctgcgttctacgtgggt
ataagaggcgcgaccagcgtcggtaccgtcgcagtcttg

# Figure 15

Analysis method for microRNA expression




# Figure 16



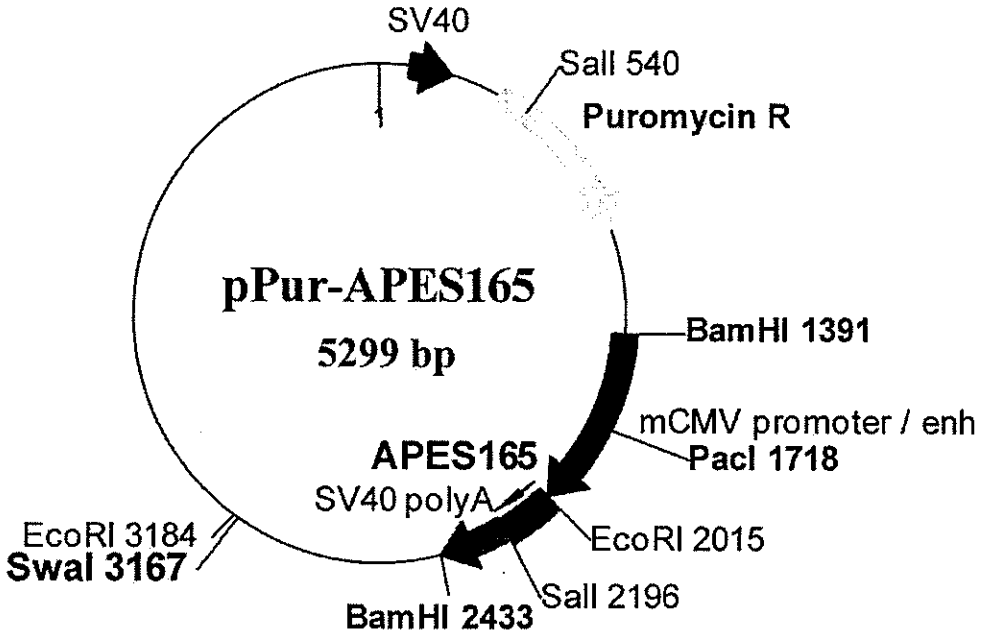

1, 2, 3 - APES 40-61 5' primer / mRQ 3' primer

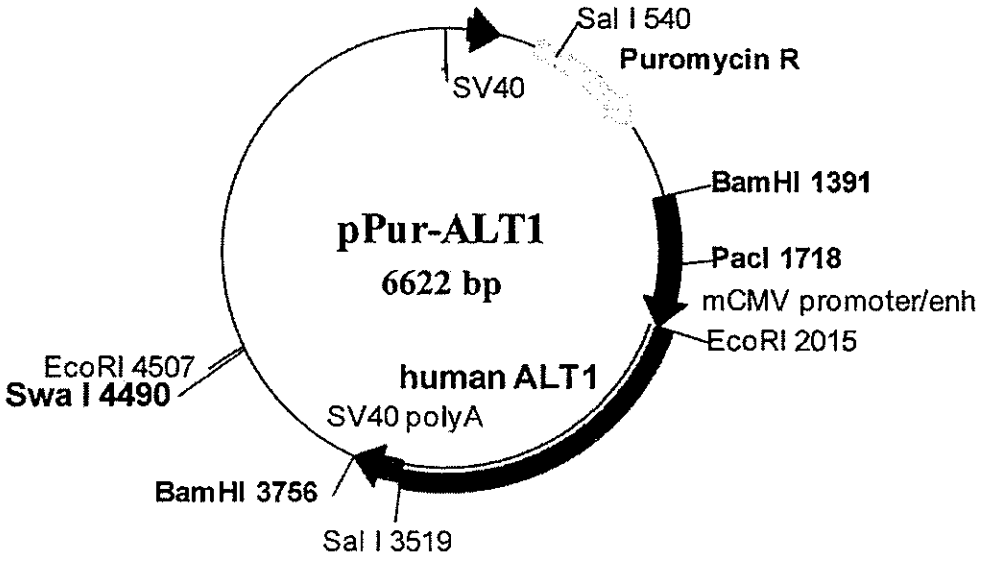
4 - U6 snRNA 5' primer / mRQ 3' primer

⇨ - microRNA (APES 40-61)

⇦ - U6 snRNA

*Figure 17*


SV40
SalI 540
Puromycin R
pPur-APES165
5299 bp
BamHI 1391
mCMV promoter / enh
PacI 1718
APES165
SV40 polyA
EcoRI 2015
EcoRI 3184
SwaI 3167
SalI 2196
BamHI 2433

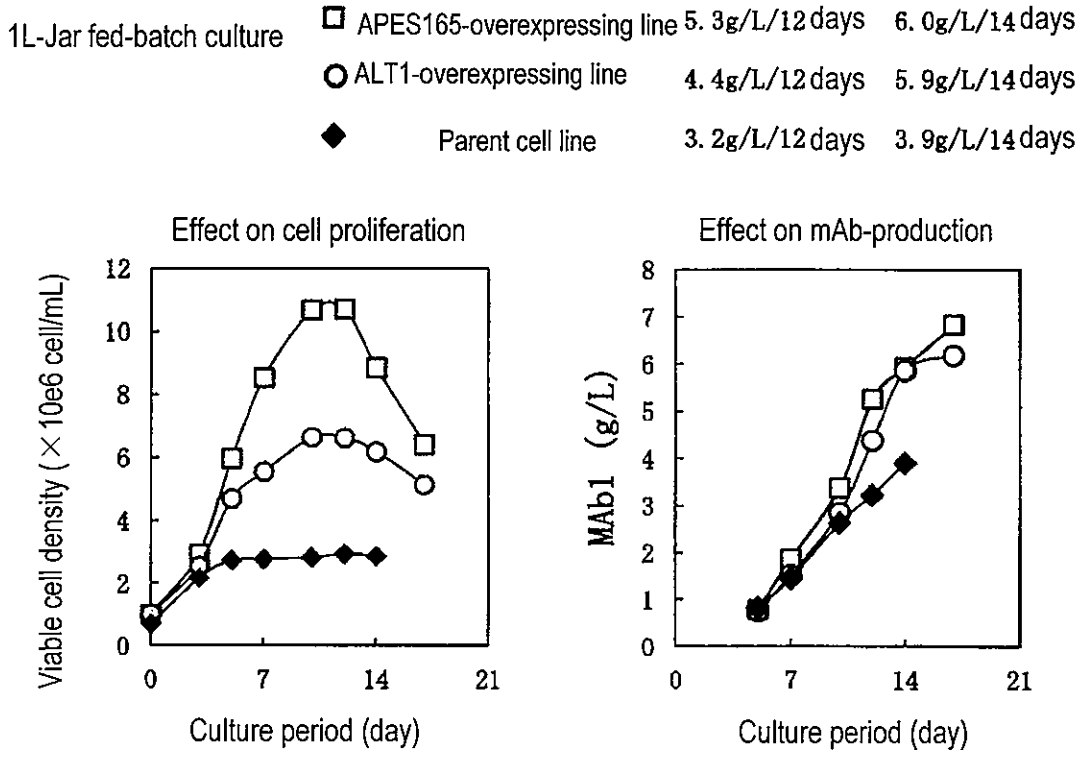

Sal I 540
Puromycin R
SV40
BamHI 1391
pPur-ALT1
6622 bp
PacI 1718
mCMV promoter/enh
EcoRI 2015
EcoRI 4507
Swa I 4490
human ALT1
SV40 polyA
BamHI 3756
Sal I 3519

# Figure 18

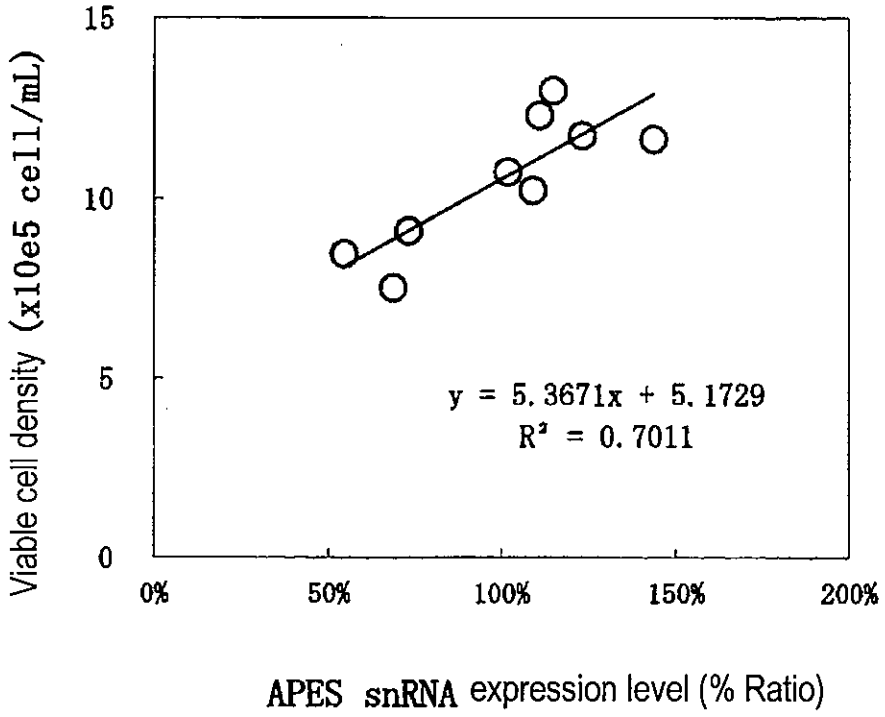
| MAb1-producing cell line | Maximum viable cell density (x10e6 cells/mL) | mAb yield (g/L/14 days) |
|---|---|---|
| APES165-overexpressing cell line | 11.5 ±1.7 | 4.4±0.6 |
| ALT1-overexpressing cell line | 8.9 ±1.8 | 4.0±0.6 |
| Parent cell line | 4.1 | 3.4 |

Shaker fed-batch culture (n=3)

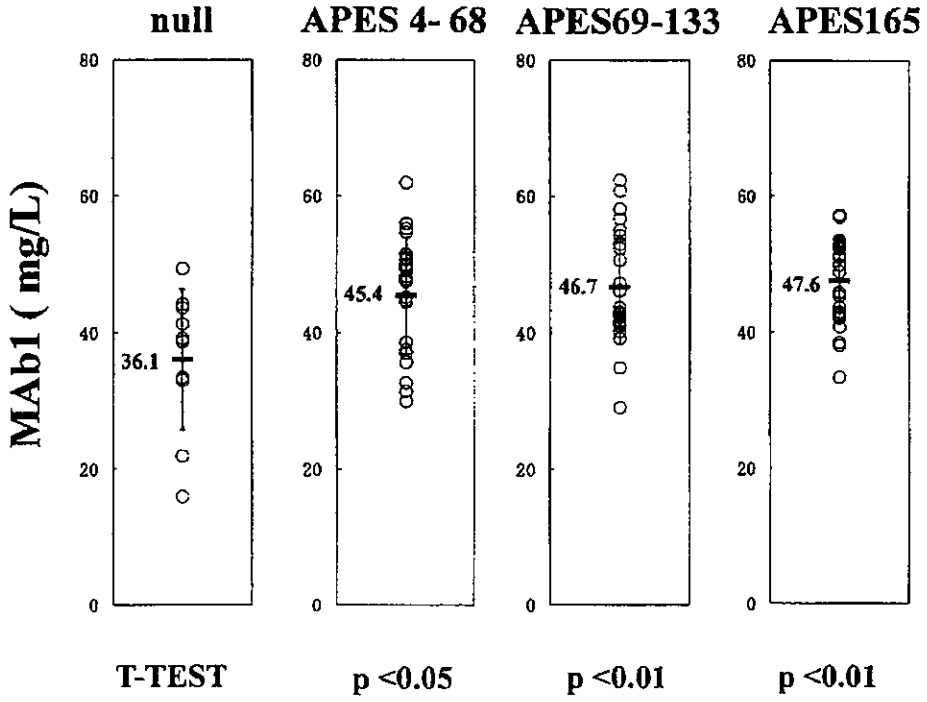
# Figure 19



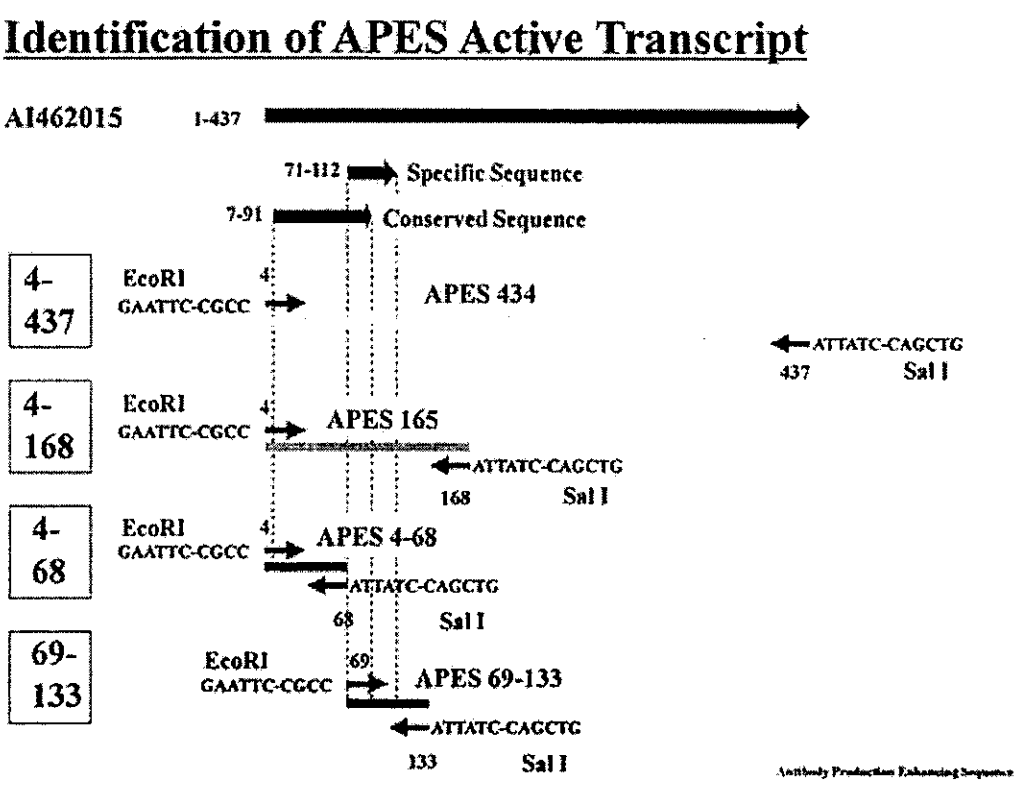
# Figure 20



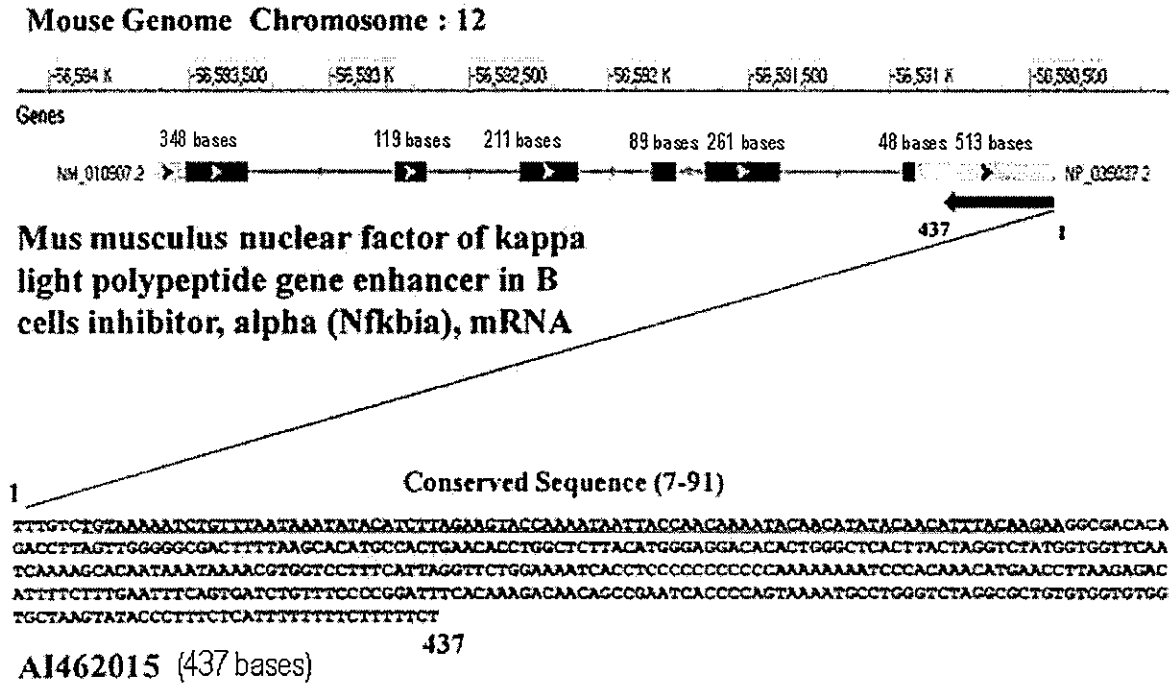

# Figure 21



# Figure 22




# Figure 23



# Figure 24

```
>gb|JH001217.1| Query1 on Cricetulus griseus cell line CHO-K1 unplaced genomic scaffold
scaffold4235, whole genome shotgun sequence Length=486540 Score = 378 bits (418), Expect = 3e-102
Identities = 352/440 (80%), Gaps = 33/440 (7%) Strand=Plus/Minus

Query 3      TGTCTGTAAAAATCTGTTTAATAAATATACATCTTAGAAGTACCAAAATAATTACCAACA 62      (AI462015)
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 385673 TGTCTGTAAAAATCTGTTTAATAAATATACATCTTAGAAGTACCAAAATAATTACCAACA 385614 (CHO-K1 genome)

Query 63     AAATACAACATATACAACATTTACAAGAAGGCGACACAGACCTTAGTTGGGGGCGACTTT 122     (AI462015)
             ||||||| ||||||||||||||||||||| || ||| | |||| ||| ||| | ||||
Sbjct 385613 AAATACACCATATACAACATTTACAAGAGGGTAACAAAAACCTCAGTCGGGAGTGACT-- 385556 (CHO-K1 genome)

Query 123    TAAGCACATGCCACTGAACACCTGGCTCTTACATGGGAGGACACACTGGGCTCACTTACT 182     (AI462015)
             ||||||| ||||| |||||||| || |||||| |||||| || |||||| |||
Sbjct 385555 --AGCACATACCACTCAACACCTGGTTC-TACATGTGAGGACATACCAGGCTCAGCTACC 385499 (CHO-K1 genome)

Query 183    AGGTCTATGGTGGTTCAATCAAAAGCACAATAAATAAAACGTGGT-CCTTTCATTAGGTT 241     (AI462015)
             || |||| ||||| ||||||||||||||||||| || ||||| |||||||| || |
Sbjct 385498 AGATCTA---CCGTTCAGTCAAAAGCACAATAAATAGAATGTGGTCCCTTTCATCAG--T 385444 (CHO-K1 genome)

Query 242    CTGGAAAATCACCTccccccccccaaaaaaaTCCCACAAACATGAACCTTAAGAGACA 301     (AI462015)
             |||||||| |||||||| |||| | ||| || ||| | |||| |||||
Sbjct 385443 CTGGAAAACCACCTCCC----------AAAACCTCACGAATGTGAGCTTTAAAAGACA 385396 (CHO-K1 genome)

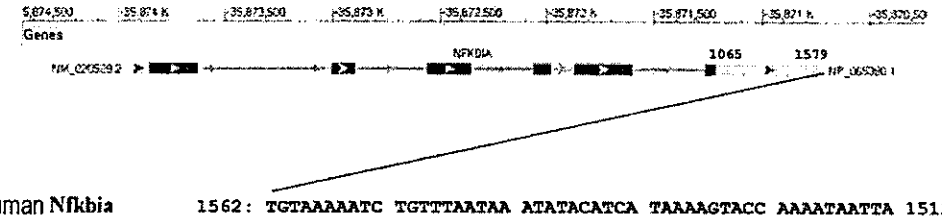
Query 302    TTTTCTTTGAATTTCAGTGATCTGTTTCCCCGGATTTCACAAAGACAACA----GCCGAA 357     (AI462015)
             |||||||||||||| || ||||||||||||||| |||||||||| | |||| ||| |
Sbjct 385395 TTTTCTTTGAATTCCAATGATCTGTTTCCCC--ATTTCACAAAAATAACAATCTGCC--A 385340 (CHO-K1 genome)

Query 358    TCACCCCAGTAAAATGCCTGGGTCTAGGCGCTGTGTGGTGTGGTGCTAAGTATA-CCCTT 416     (AI462015)
             ||||| |||||| |||| |||| |||| |||| |||||||| |||||||| |||||
Sbjct 385339 TCACCAGAGTAAGATGCTTGGGGGCAGGCTGTGTGCAGTGTGGTGGTAAGTATATCCCTT 385280 (CHO-K1 genome)

Query 417    TC-TCAttttttttattttt 435
             || |  ||||||||||| ||
Sbjct 385279 TCTTTCTTTTTTTTCTTCTT 385260
```

# Figure 25-1

(a)

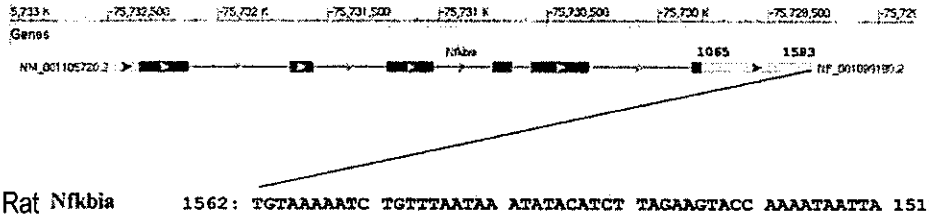



```
Human Nfkbia      1562: TGTAAAAATC TGTTTAATAA ATATACATCA TAAAAGTACC AAAATAATTA 1511
                        ********** ********** *********  ** ******* **********
AI462015             7: TGTAAAAATC TGTTTAATAA ATATACATCT TAGAAGTACC AAAATAATTA   56

                  1512: CCAACAA--T ACATTATGTA CACCATTTAC AGGAG 1478
                        *******  * ***  ** ** ** ******* * **
                    57: CCAACAAAAT ACAACATATA CAACATTTAC AAGAA   91
```

Matching = 75/85 (88%)

(b)



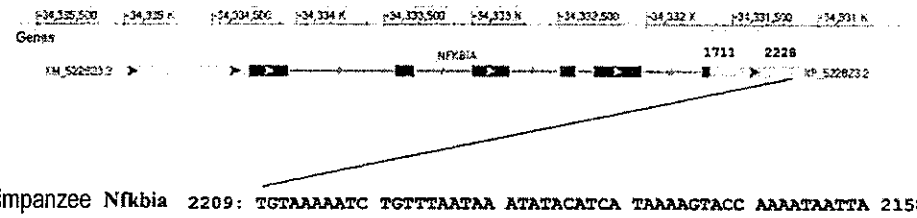

```
Rat Nfkbia        1562: TGTAAAAATC TGTTTAATAA ATATACATCT TAGAAGTACC AAAATAATTA 1511
                        ********** ********** ********** ********** **********
AI462015             7: TGTAAAAATC TGTTTAATAA ATATACATCT TAGAAGTACC AAAATAATTA   56

                  1512: CCAACAAAAT ACACCATATA CAACATTTAC AAGAA 1478
                        ********** *** ****** ********** *****
                    57: CCAACAAAAT ACAACATATA CAACATTTAC AAGAA   91
```

Matching = 84/85 (99%)

# Figure 25-2

(c)




Chimpanzee Nfkbia 2209: TGTAAAAATC TGTTTAATAA ATATACATCA TAAAAGTACC AAAATAATTA 2158

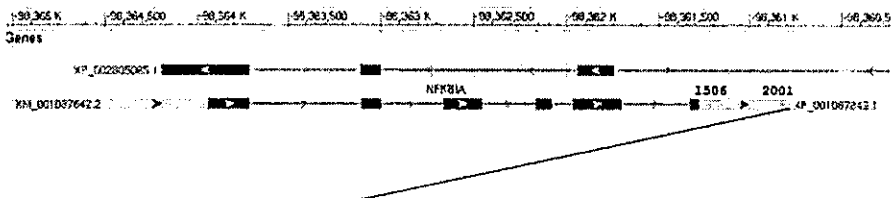
AI462015 7: TGTAAAAATC TGTTTAATAA ATATACATCT TAGAAGTACC AAAATAATTA 56

2159: CTAAC--AAT ACATTATGTA CATCATTTAC AGGAGGGTAA C 2121

57 : CCAACAAAAT ACAACATATA CAACATTTAC A--AG----A A 91

Matching = 75/85 (88%)

(d)




Rhesus monkey Nfkbia 1995: TGGAAAAATC TGTTTAATAA ATATACATAA TAAAAGTACC AAAATAATTA 1944

AI462015 7: TGTAAAAATC TGTTTAATAA ATATACATCT TAGAAGTACC AAAATAATTA 56

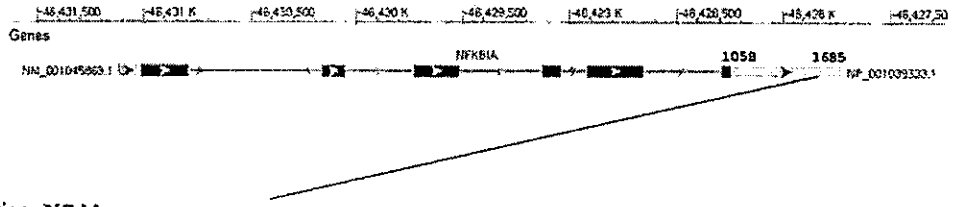
1945: CCAAC--AAT ACACTATGTA CACCATTTAC AGAAGGGTAA C 1907

57: CCAACAAAAT ACAACATATA CAACATTTAC --AAG----A A 91

Matching = 74/85 (87%)

# Figure 25-3

(e)




Bovine Nfkbia 1570: TGTAAAAATC TGTTTAATAA ATATACATCT TAAAAGTACC AAAATAATTA 1519

AI462015 7: TGTAAAAATC TGTTTAATAA ATATACATCT TAGAAGTACC AAAATAATTA 56

1520: CTGACAAAAT ACACTATGTA CACTATTTAC AGGAGGGGAA 1481

57: CCAACAAAAT ACAACATATA CAACATTTAC A-----AGAA 91

**Matching = 76/85 (89%)**

Figure 26

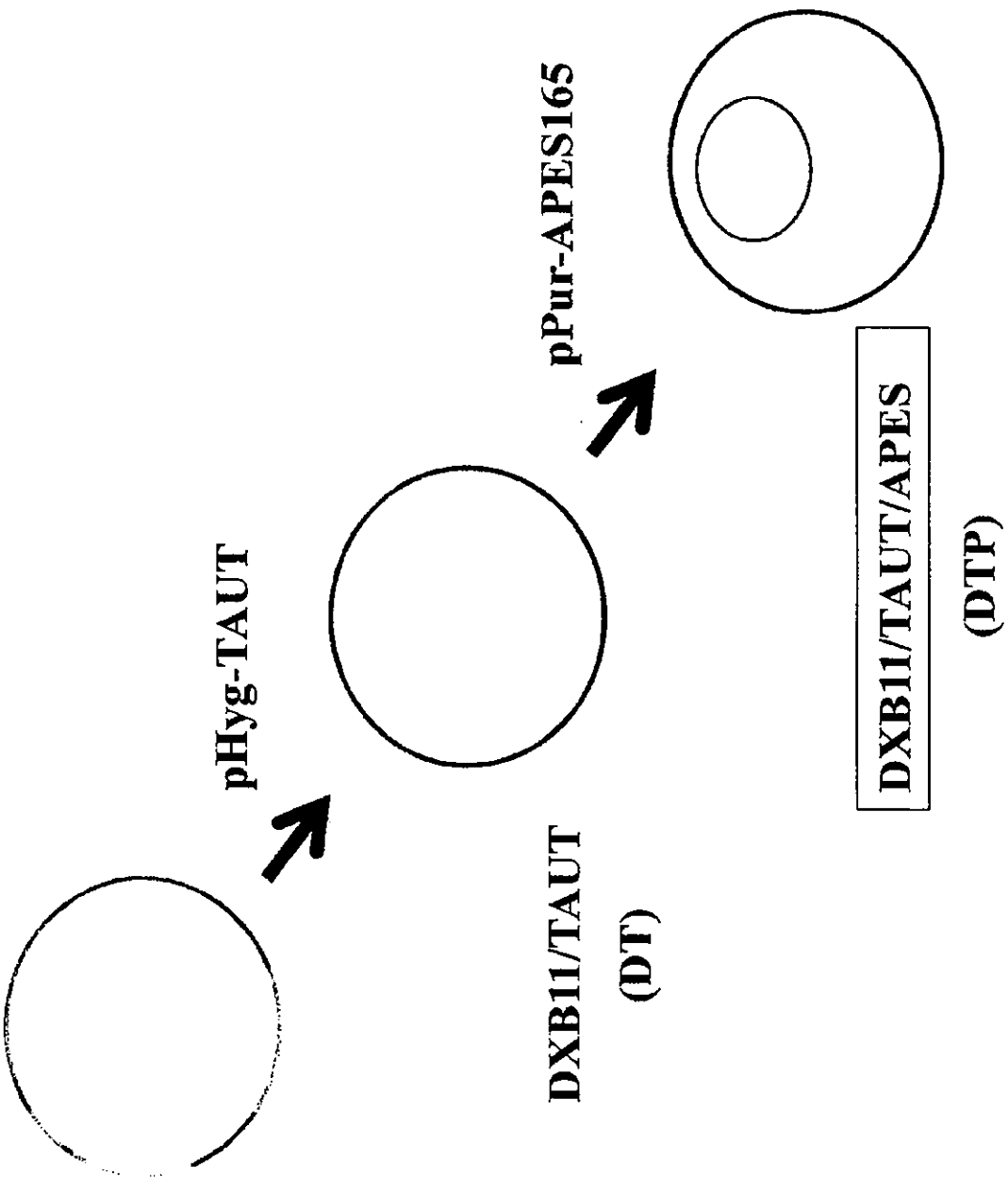
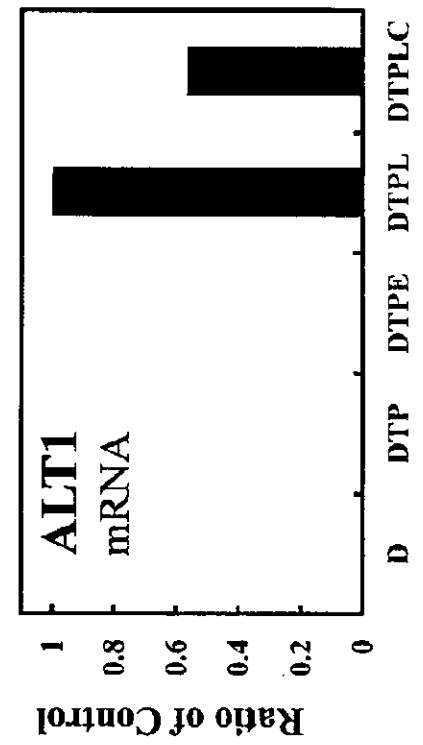
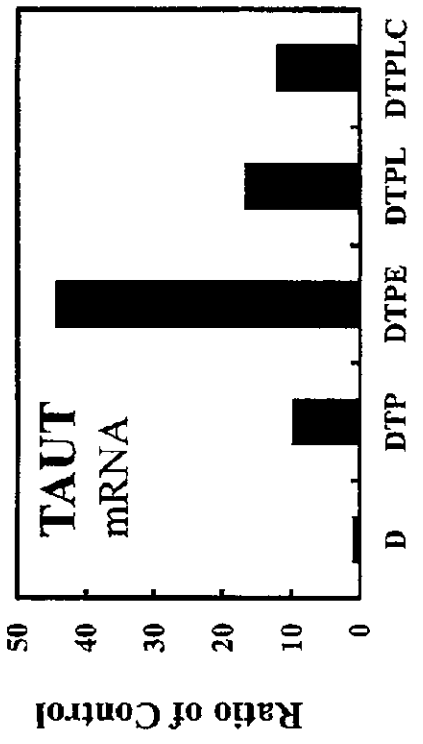

DXB11
pHyg-TAUT
DXB11/TAUT
(DT)
pPur-APES165
DXB11/TAUT/APES
(DTP)

Figure 27


ALT1
mRNA
Ratio of Control
1
0.8
0.6
0.4
0.2
0
D
DTP
DTPE
DTPL
DTPLC


TAUT
mRNA
Ratio of Control
50
40
30
20
10
0
D
DTP
DTPE
DTPL
DTPLC

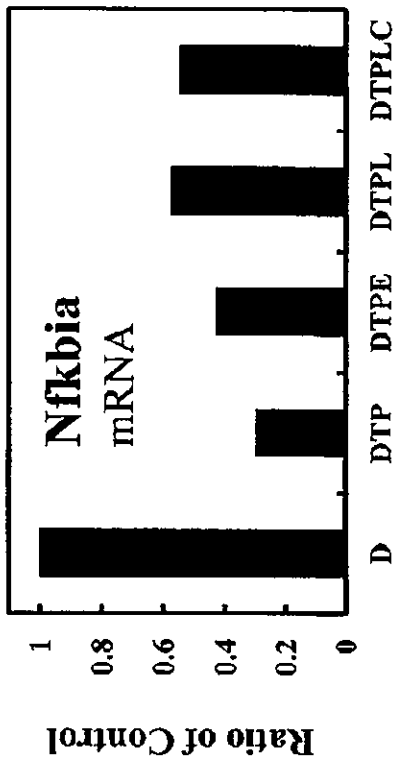

Nfkbia
mRNA
Ratio of Control
1
0.8
0.6
0.4
0.2
0
D
DTP
DTPE
DTPL
DTPLC

Figure 28

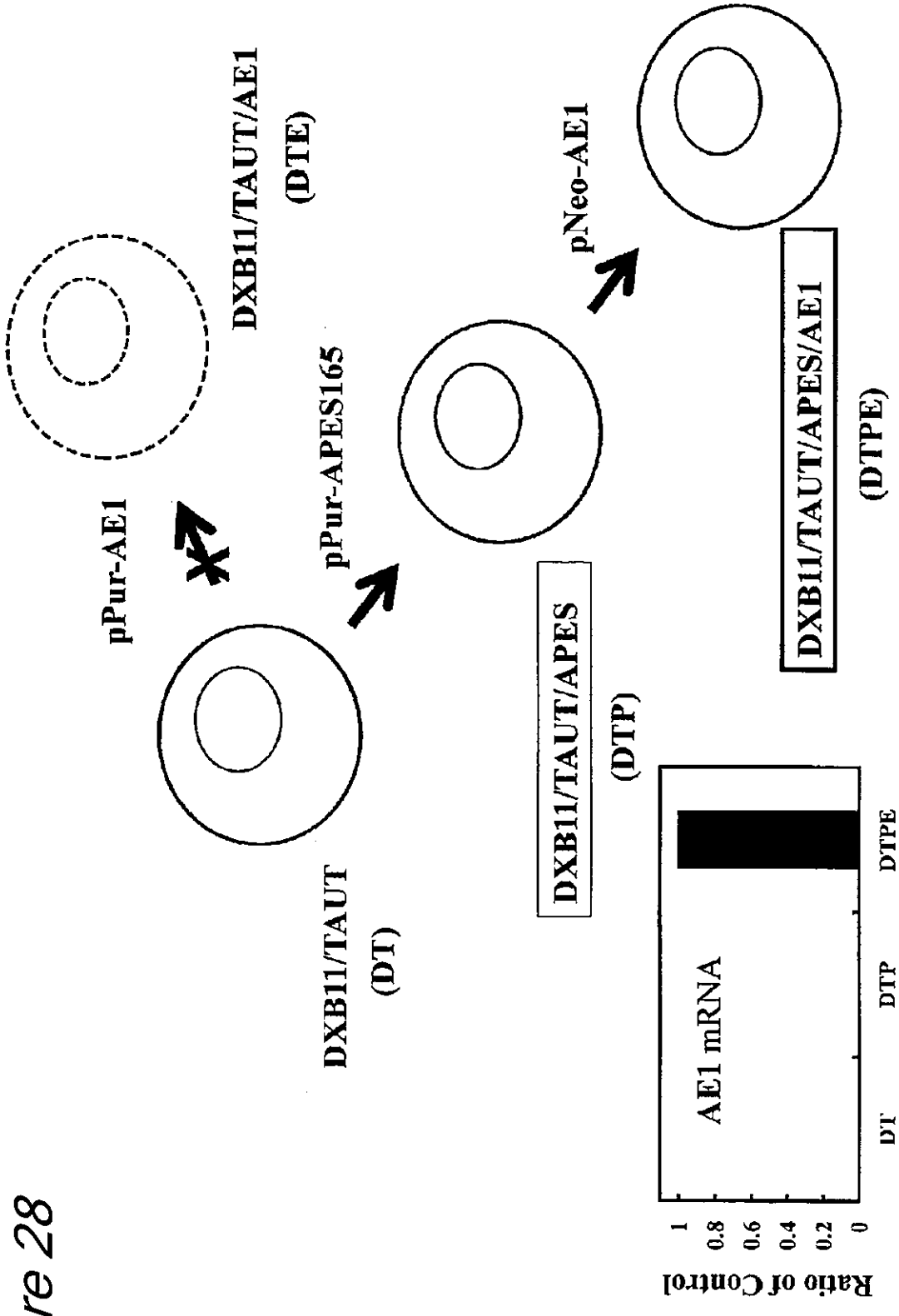

pPur-AE1
DXB11/TAUT
(DT)
pPur-APES165
DXB11/TAUT/APES
(DTP)
pNeo-AE1
DXB11/TAUT/APES/AE1
(DTPE)
DXB11/TAUT/AE1
(DTE)
AE1 mRNA
Ratio of Control
0
0.2
0.4
0.6
0.8
1
DT
DTP
DTPE

Figure 29

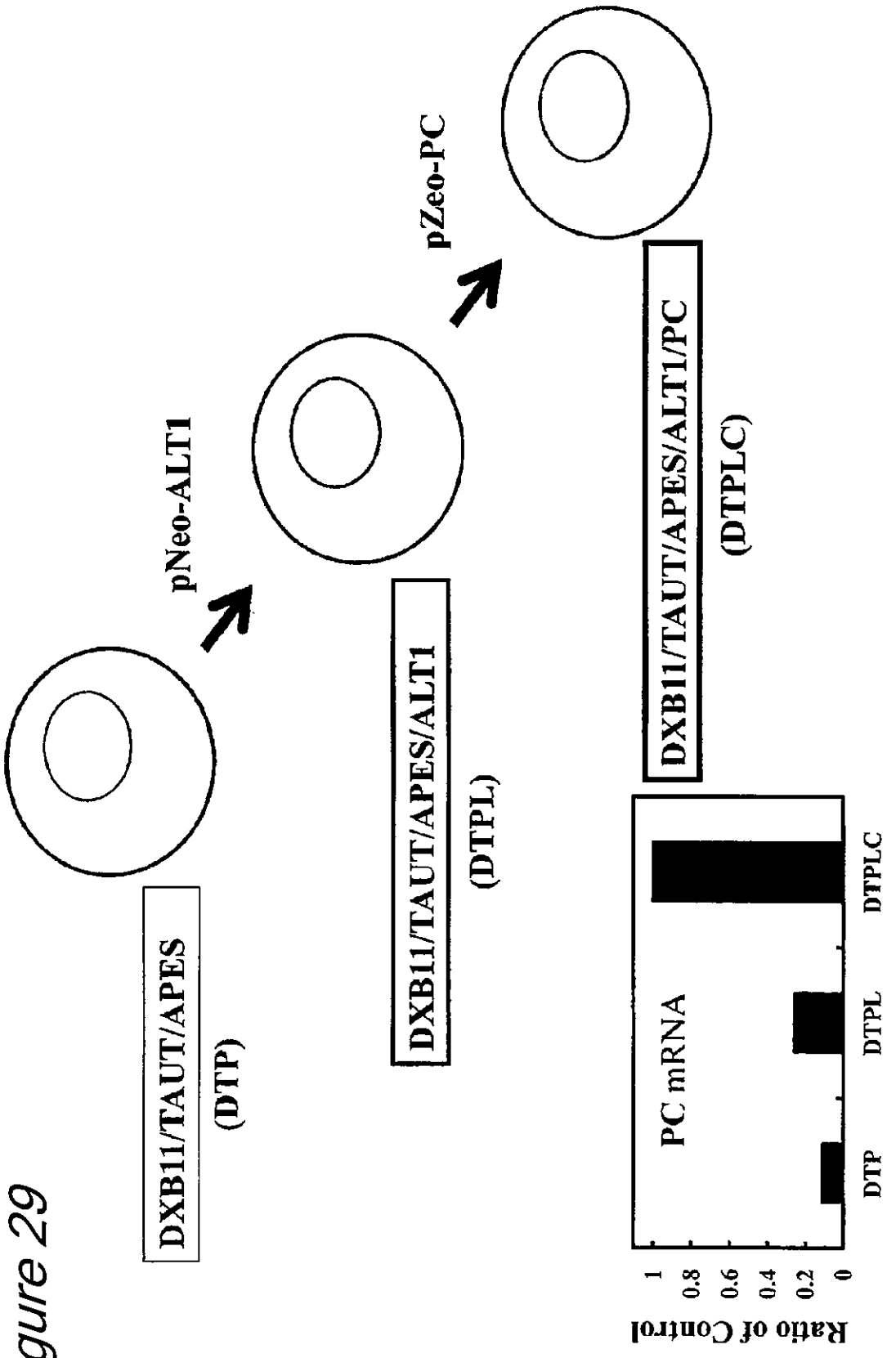

DXB11/TAUT/APES
(DTP)
pNeo-ALT1
DXB11/TAUT/APES/ALT1
(DTPL)
pZeo-PC
DXB11/TAUT/APES/ALT1/PC
(DTPLC)
PC mRNA
Ratio of Control
0
0.2
0.4
0.6
0.8
1
DTP
DTPL
DTPLC

Figure 30

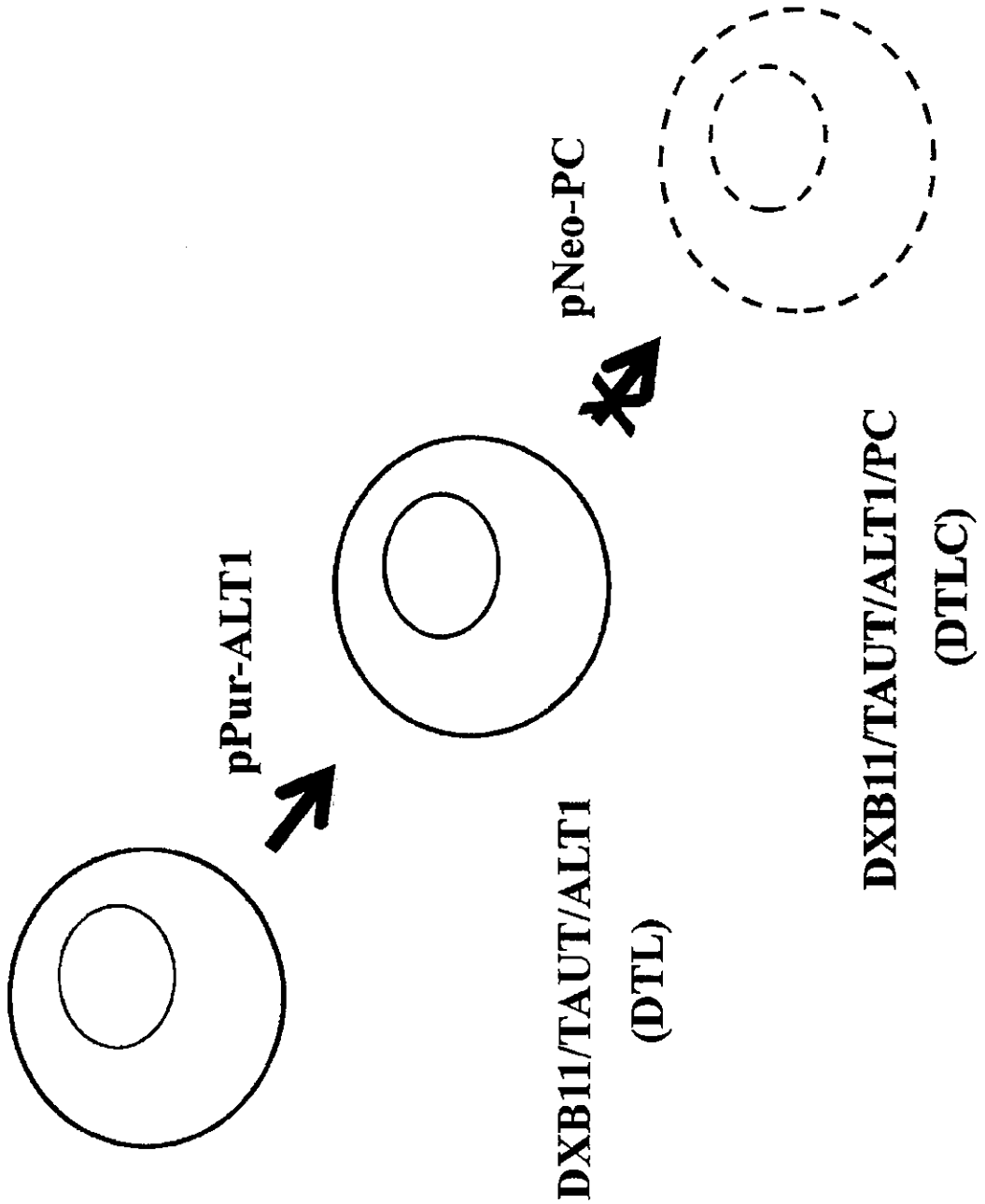

DXB11/TAUT
(DT)
pPur-ALT1
DXB11/TAUT/ALT1
(DTL)
pNeo-PC
DXB11/TAUT/ALT1/PC
(DTLC)

*Figure 31*

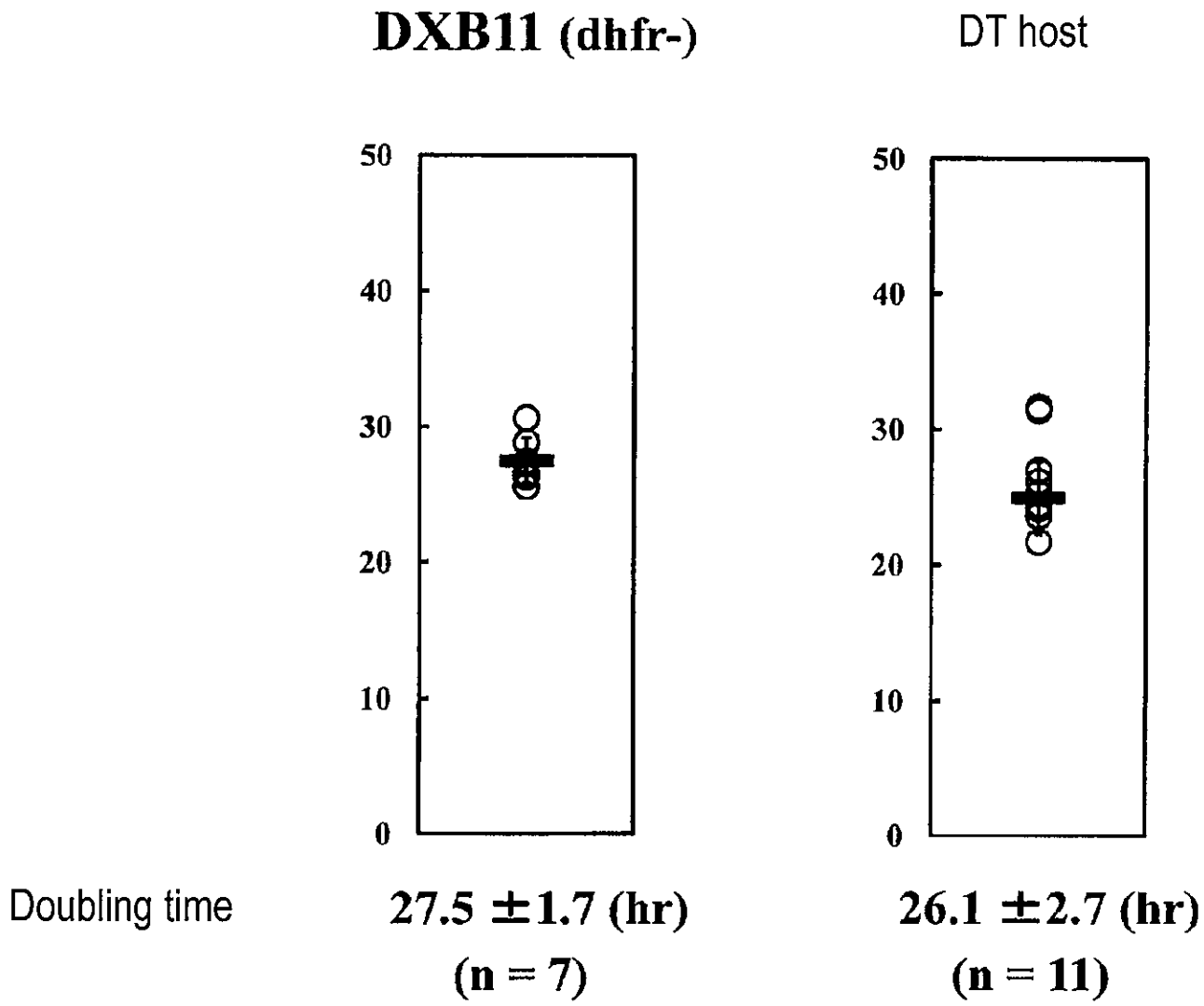

DXB11 (dhfr-)
DT host
50
40
30
20
10
0
50
40
30
20
10
0
Doubling time
27.5 ±1.7 (hr)
(n = 7)
26.1 ±2.7 (hr)
(n = 11)

Figure 32

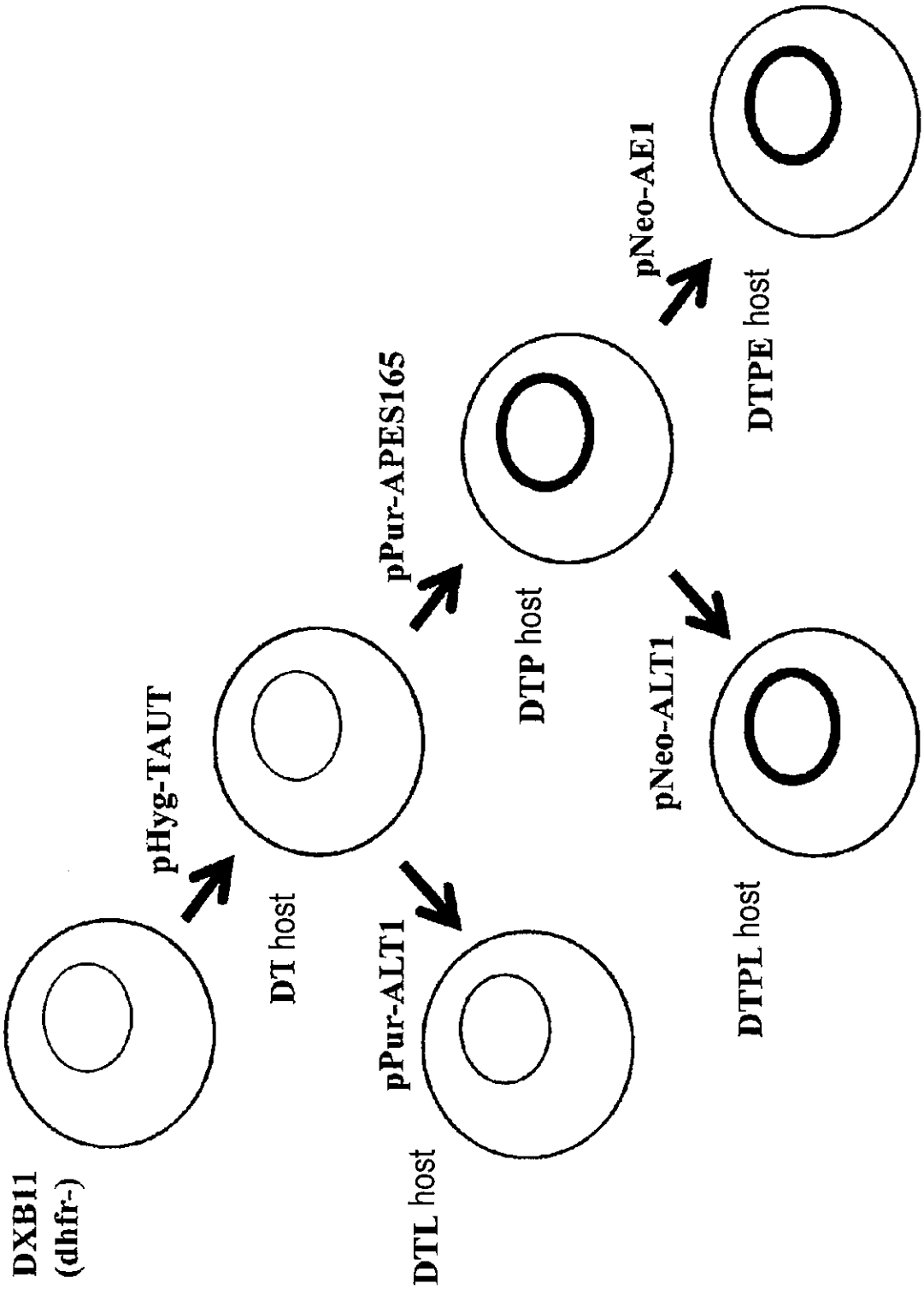
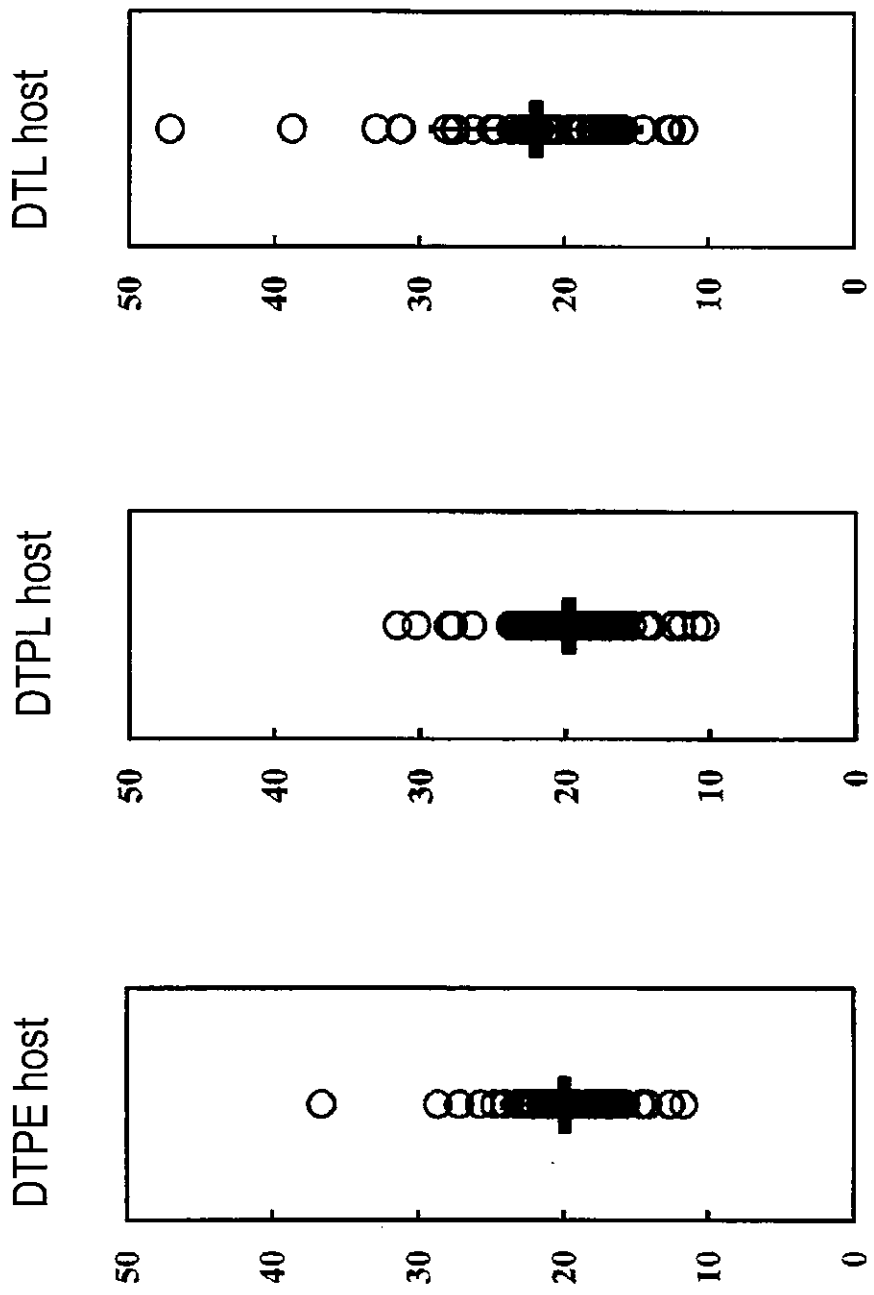

DXB11
(dhfr-)
pHyg-TAUT
DT host
pPur-ALT1
DTL host
pPur-APES165
DTP host
pNeo-ALT1
DTPL host
pNeo-AE1
DTPE host

*Figure 33*


DTPE host
DTPL host
DTL host
50
40
30
20
10
0
Doubling time
19.9 ±4.3 (hr)
(n = 57)
19.8 ±4.3 (hr)
(n = 57)
21.9 ±7.3 (hr)
(n = 57)