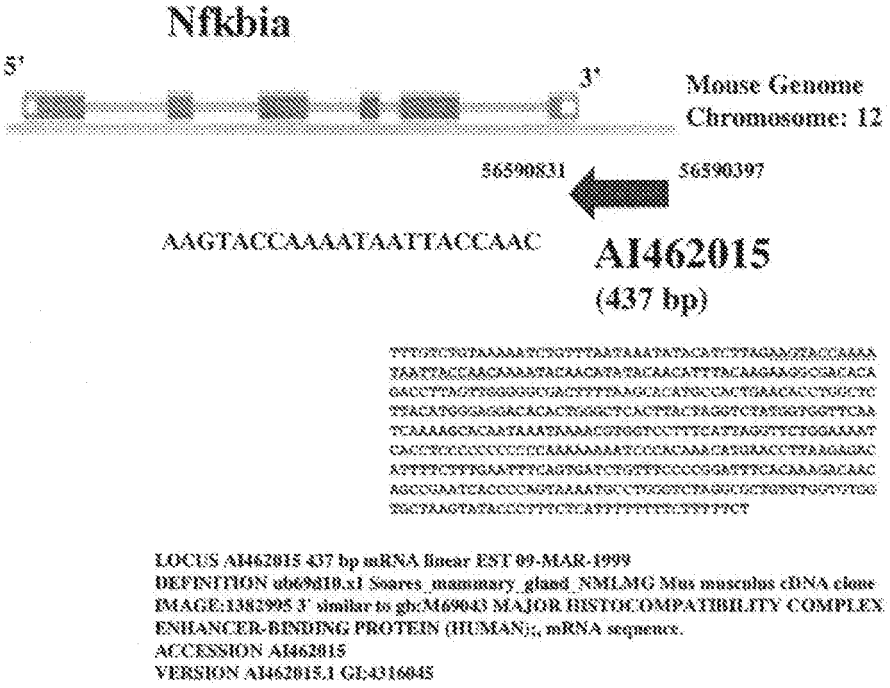
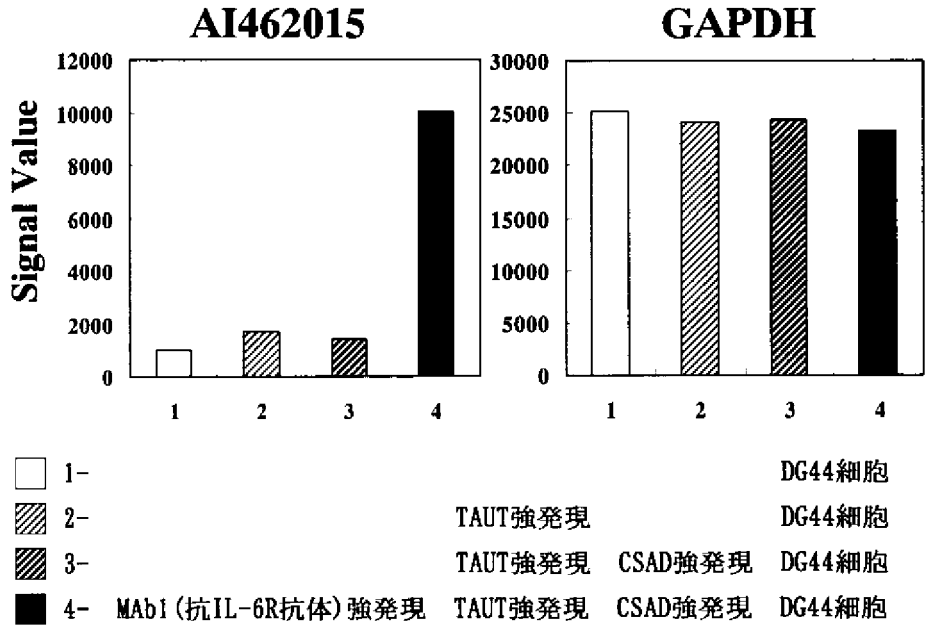
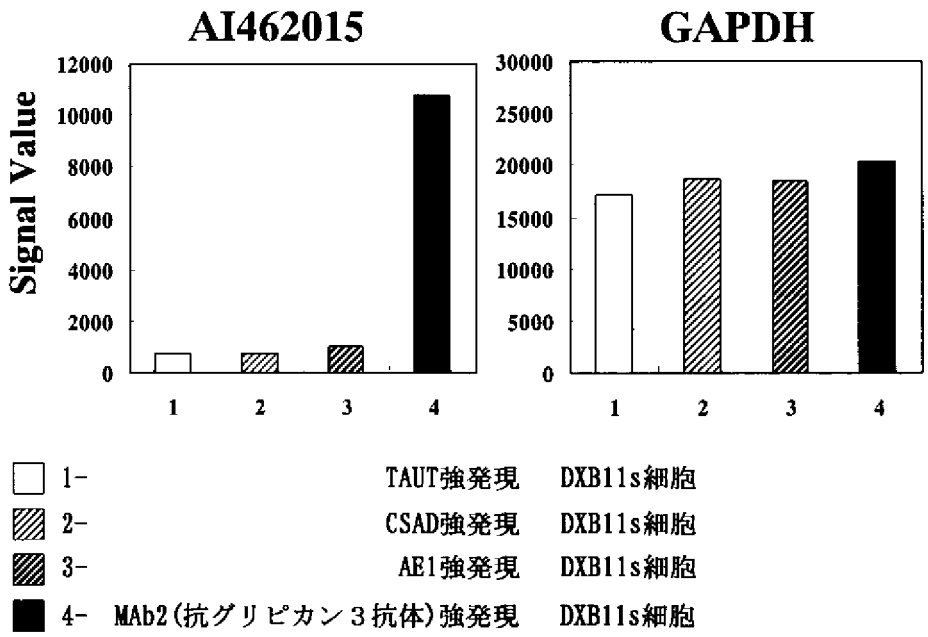
[図1]


Nfkbia
5'
3'
Mouse Genome
Chromosome: 12
56590831
56590397
AAGTACCAAAATAATTACCAAC
AI462015
(437 bp)
LOCUS AI462815 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 AI462815
VERSION AI462815.1 GI:4316845

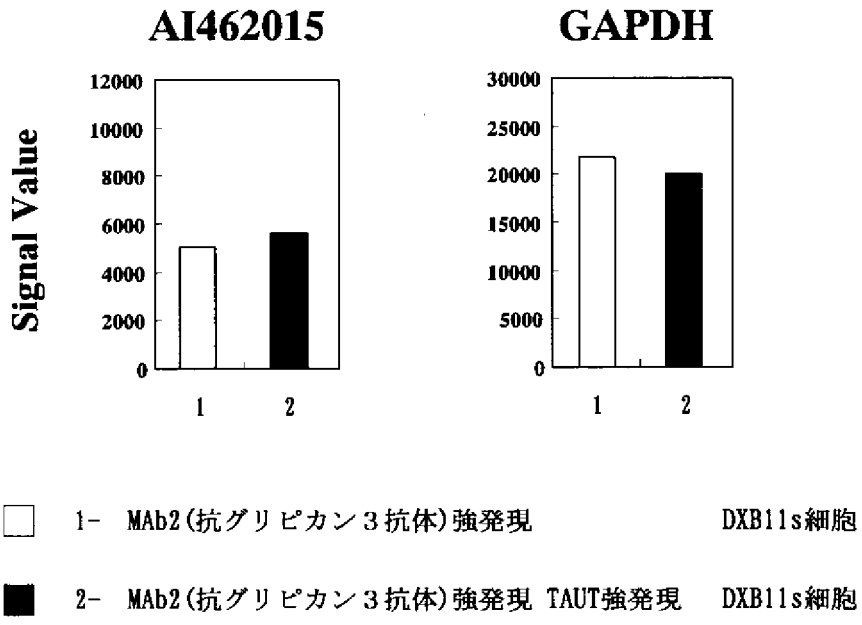
[図2]


AI462015
GAPDH
Signal Value
12000
10000
8000
6000
4000
2000
0
30000
25000
20000
15000
10000
5000
0
1
2
3
4
1- DG44細胞
2- TAUT強発現 DG44細胞
3- TAUT強発現 CSAD強発現 DG44細胞
4- MAb1(抗IL-6R抗体)強発現 TAUT強発現 CSAD強発現 DG44細胞

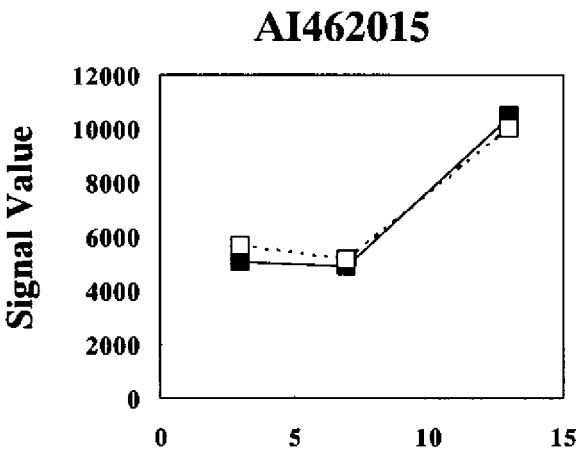
[図3]


AI462015
GAPDH
Signal Value
12000
10000
8000
6000
4000
2000
0
30000
25000
20000
15000
10000
5000
0
1
2
3
4
1- TAUT強発現 DXB11s細胞
2- CSAD強発現 DXB11s細胞
3- AE1強発現 DXB11s細胞
4- MAb2(抗グリピカン３抗体)強発現 DXB11s細胞

[図4]


AI462015
GAPDH
Signal Value
12000
10000
8000
6000
4000
2000
0
30000
25000
20000
15000
10000
5000
0
1
2
1- MAb2(抗グリピカン３抗体)強発現 DXB11s細胞
2- MAb2(抗グリピカン３抗体)強発現 TAUT強発現 DXB11s細胞

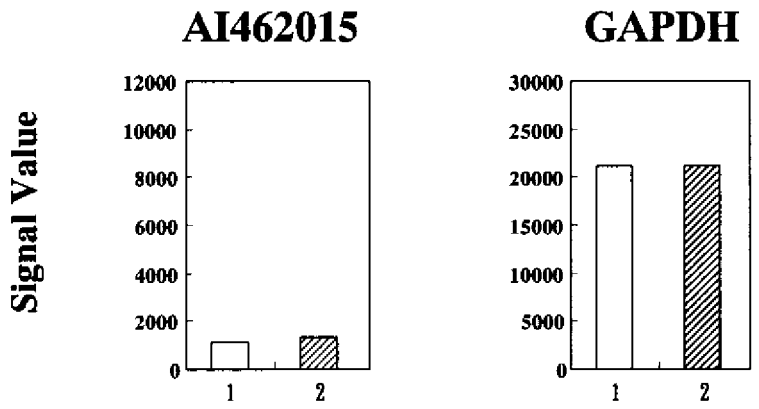
[図5]



■ 1- MAb2(抗グリピカン３抗体)強発現 DXB11s細胞

□ 2- MAb2(抗グリピカン３抗体)強発現 TAUT強発現 DXB11s細胞

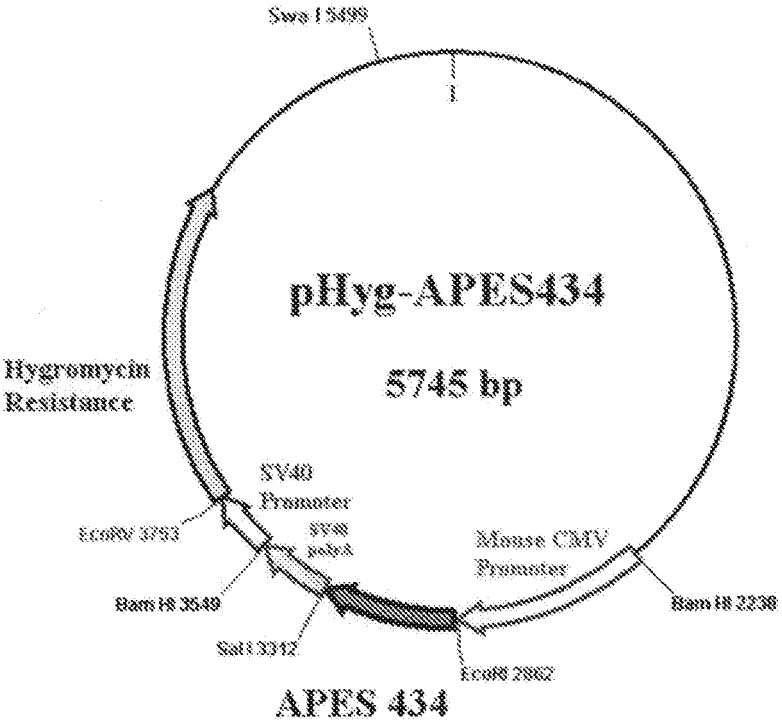
[図6]



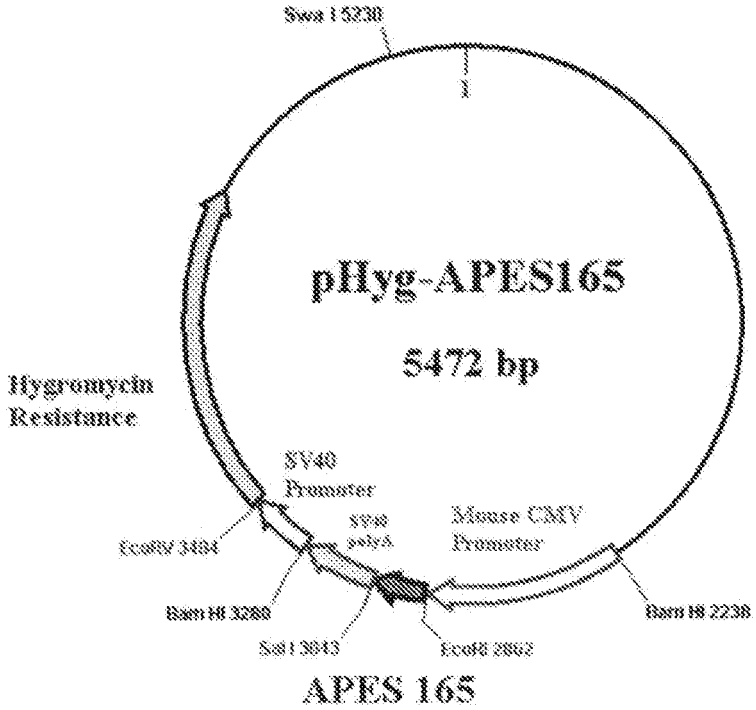
□ 1- MAb1(抗IL-6R抗体)強発現 DXB11s細胞 (Hy-Fish 添加)

▨ 2- MAb1(抗IL-6R抗体)強発現 DXB11s細胞 (Porcine Lysate 添加)

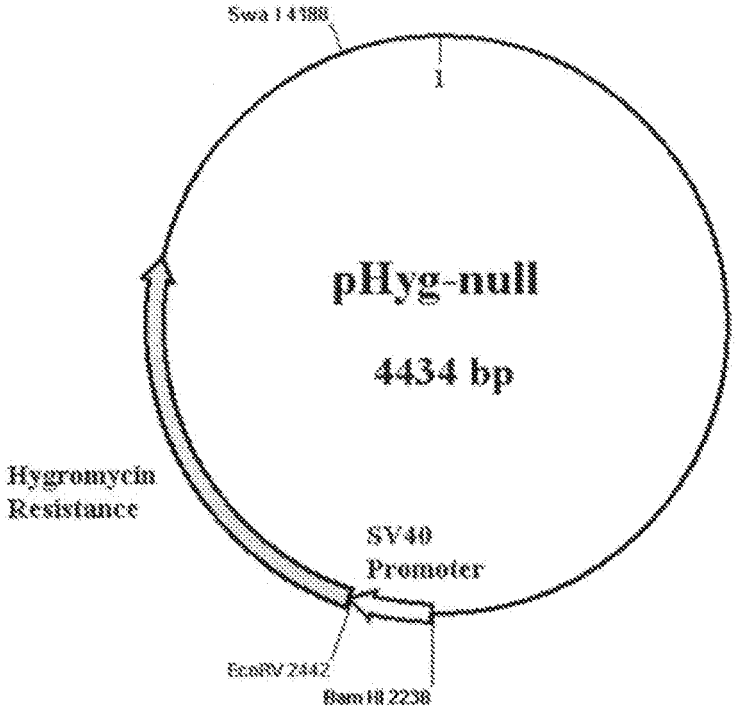
[図7]


Swa I 5499
1
pHyg-APES434
5745 bp
Hygromycin
Resistance
SV40
Promoter
EcoRV 3753
SV40
polyA
Mouse CMV
Promoter
Bam HI 3549
Bam HI 2238
Sal I 3312
EcoR I 2862
APES 434

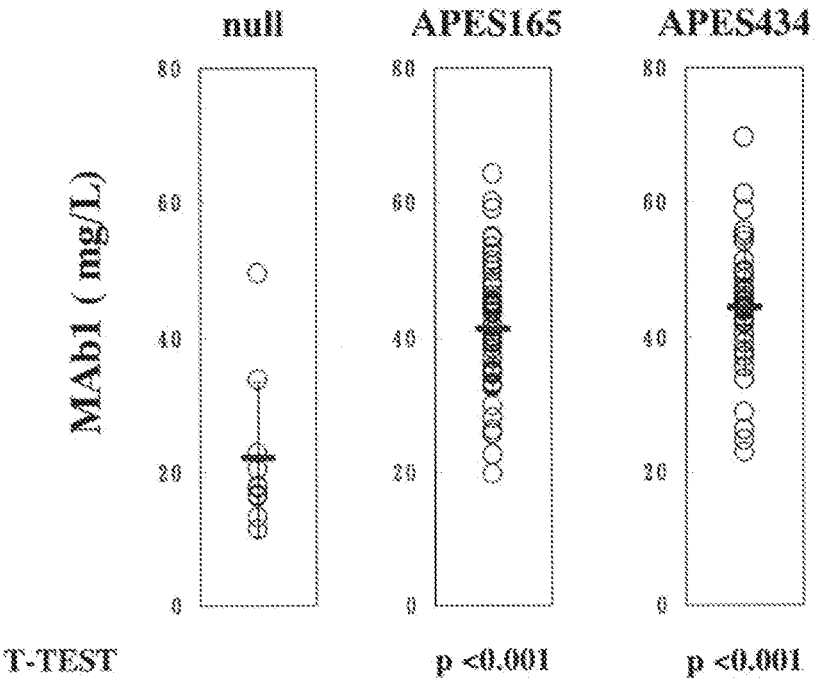
[図8]


Swa I 5230
1
pHyg-APES165
5472 bp
Hygromycin
Resistance
SV40
Promoter
EcoRV 3484
SV40
polyA
Mouse CMV
Promoter
Bam HI 3280
Bam HI 2238
Sal I 3043
EcoR I 2862
APES 165

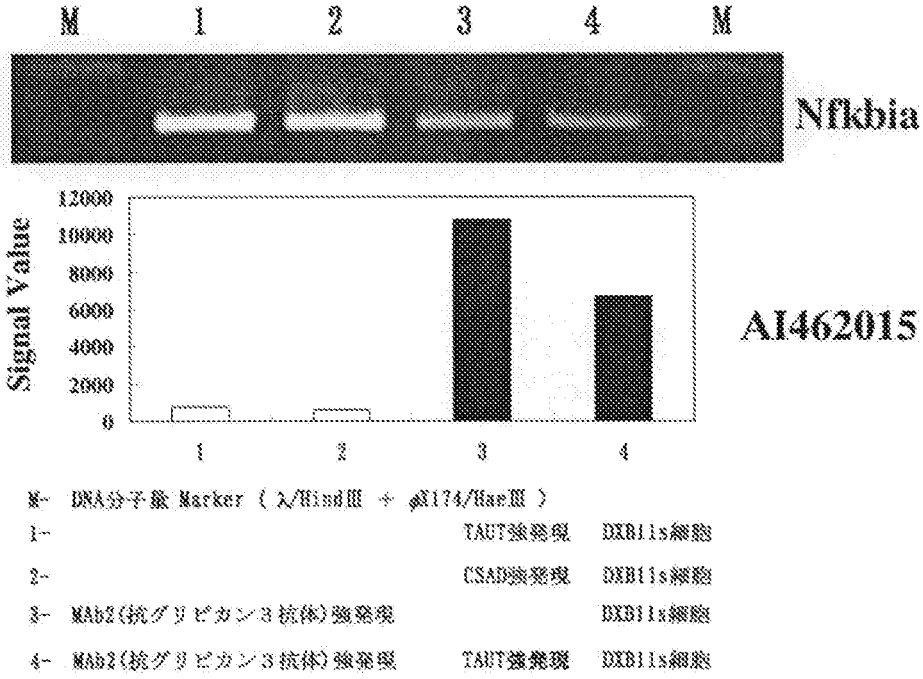
[図9]


Swa I 4186
1
pHyg-null
4434 bp
Hygromycin
Resistance
SV40
Promoter
EcoRV 2442
Bam HI 2238

[図10]


null
APES165
APES434
MAb1 (mg/L)
80
60
40
20
0
T-TEST
p <0.001
p <0.001

[図11]



[図12]

A part of Hamster Nfkbia mRNA (134 bp)

```
          10         20         30         40         50
agtacccggatacagcagcagctgggccagctgacccgggaaaatcttca

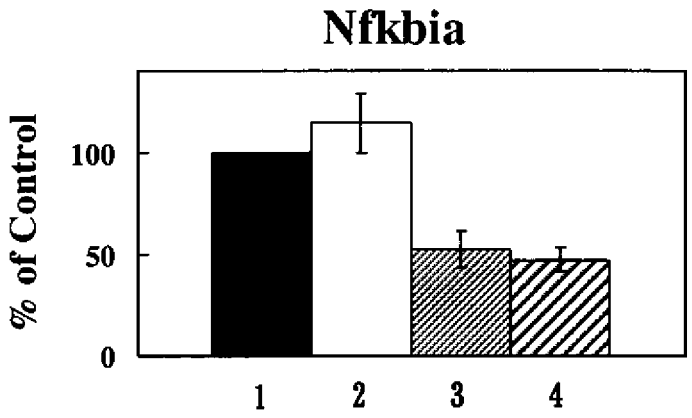
          60         70         80         90        100
gatgctgcccgagagtgaggatgaggagagctacgacacagagtcagaat

         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

[図13]

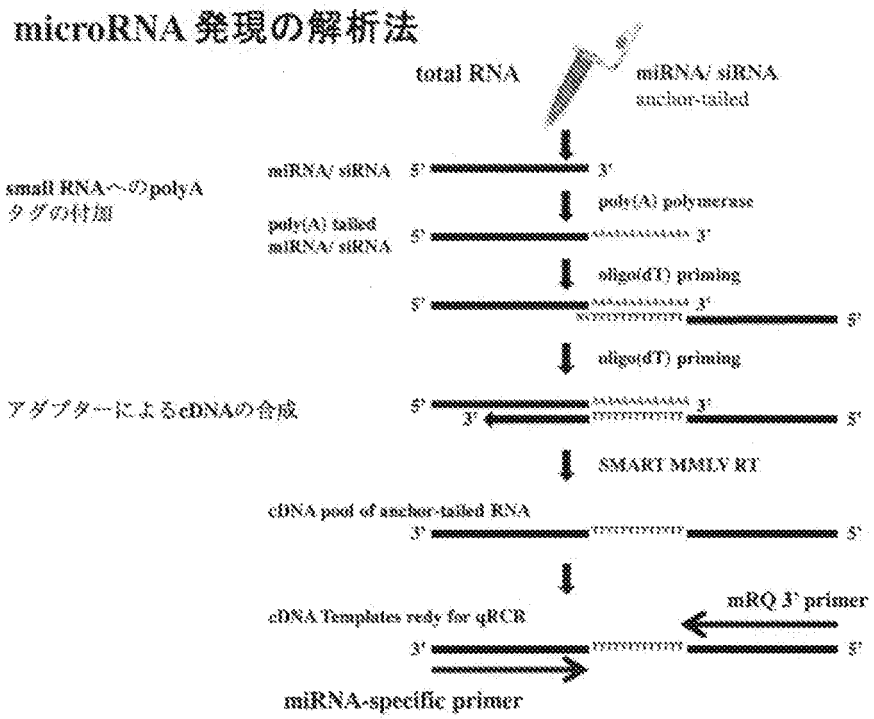


1-		TAUT強発現	DXB11s細胞
2-		CSAD強発現	DXB11s細胞
3-	MAb2(抗グリピカン３抗体)強発現		DXB11s細胞
4-	MAb2(抗グリピカン３抗体)強発現	TAUT強発現	DXB11s細胞

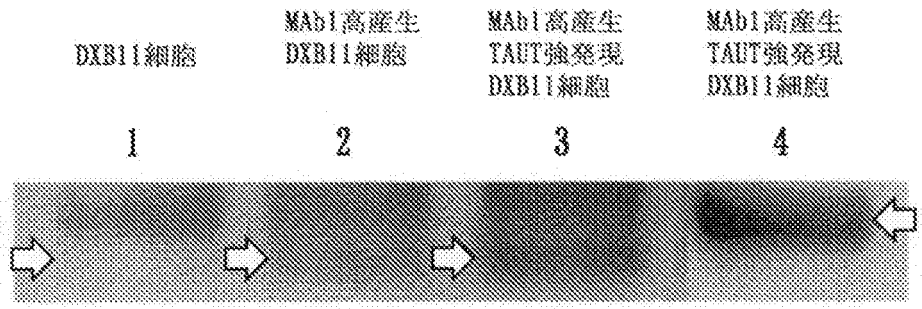
[図14]

ctctgggctcgaatggcatgggggacagctttatatggttaactccgcccgttttatgactagaaccaatagtttttaatgccaaa
tgcactgaaatcccctaatttgcaaagccaaacgcccccctatgtgagtaatacggggacttttttacccaatttcccaagcggaaa
gccccctaatacactcatatggcatatgaatcagcacggtcatgcactctaatggcggcccatagggactttccacatagggg
gcgttcaccatttcccagcataggggtggtgactcaatggcctttacccaagtacattgggtcaatgggaggtaagccaatgg
gtttttcccattactggcaagcacactgagtcaaatgggactttccactgggttttgcccaagtacattgggtcaatgggaggtga
gccaatgggaaaaacccattgctgccaagtacactgactcaatagggactttccaatgggtttttccattgttggcaagcatata
aggtcaatgtgggtgagtcaatagggactttccattgtattctgcccagtacataaggtcaatagggggtgaatcaacaggaaa
gtcccattggagccaagtacactgcgtcaatagggactttccattgggttttgcccagtacataaggtcaataggggatgagtc
aatgggaaaaacccattggagccaagtacactgactcaatagggactttccattgggttttgcccagtacataggtcaatagg
gggtgagtcaacaggaaagttccattggagccaagtacattgagtcaatagggactttccaatgggttttgcccagtacataag
gtcaatgggaggtaagccaatgggttttcccattactggcacgtatactgagtcattagggactttccaatgggttttgcccagt
acataaggtcaataggggtgaatcaacaggaaagtcccattggagccaagtacactgagtcaatagggactttccattgggttt
tgcccagtacaaaaggtcaatagggggtgagtcaatgggttttcccattattggcacgtacataaggtcaatagggggtgagtc
attgggtttttccagccaatttaattaaaacgccatgtactttcccaccattgacgtcaatgggctattgaaactaatgcaacgtga
cctttaaacggtactttcccatagctgattaatgggaaagtaccgttctcgagccaatacacgtcaatgggaagtgaaagggca
gccaaaacgtaacaccgccccggttttcccctggaaattccatattggcacgcattctattggctgagctgcgttctacgtgggt
ataagaggcgcgaccagcgtcggtaccgtcgcagtcttg

[図15]



[図16]



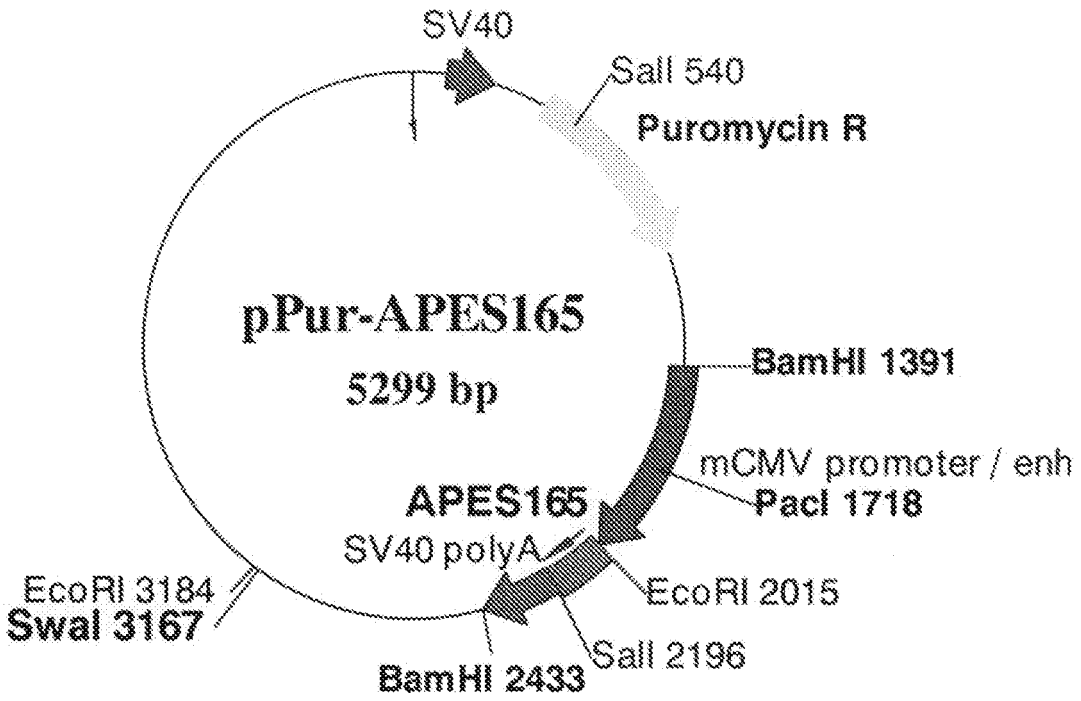
1, 2, 3 - APES 40-61 5' primer / mRQ 3' primer

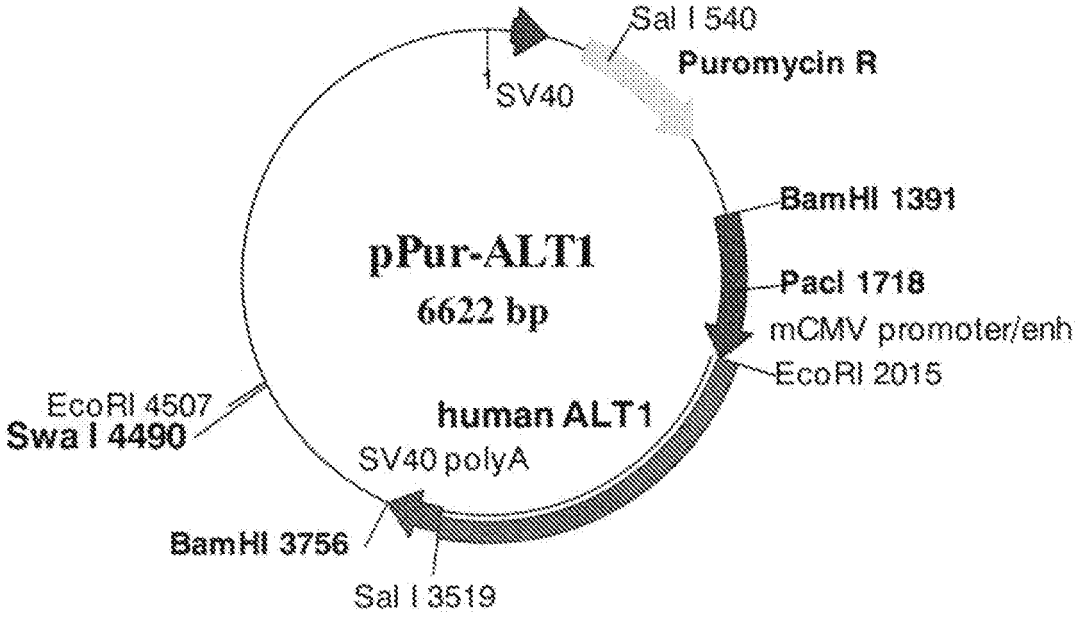
4 - U6 snRNA 5' primer / mRQ 3' primer

⇨ - microRNA (APES 40-61)

⇦ - U6 snRNA

[図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

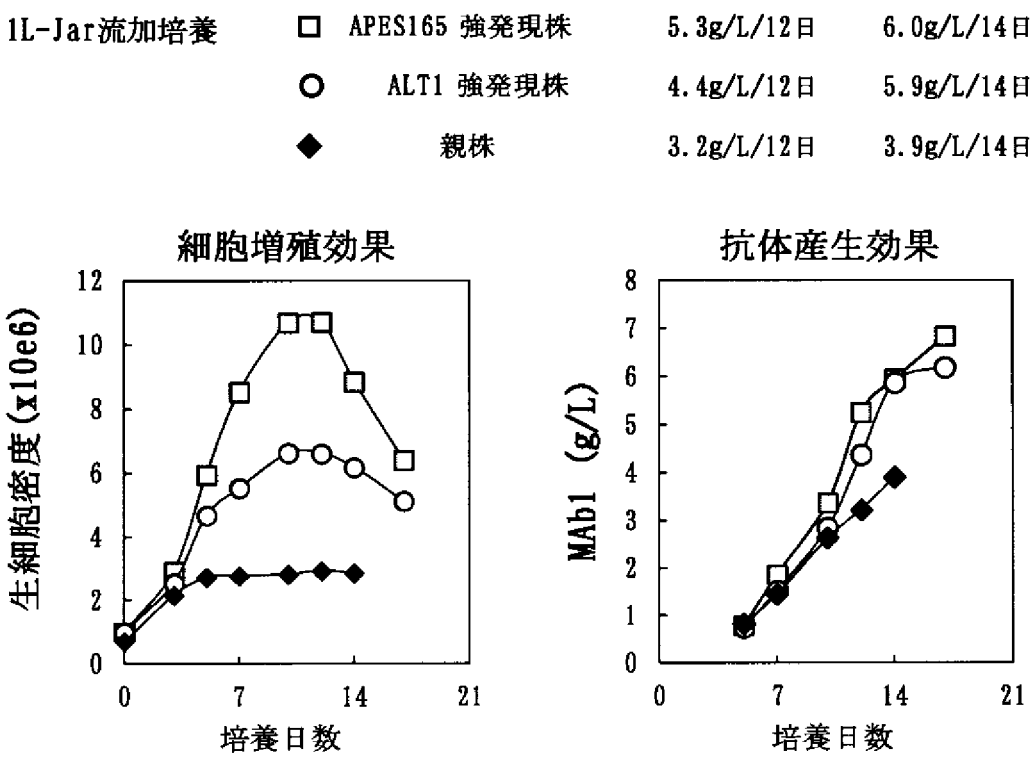

Sal I 540
Puromycin R
SV40
BamHI 1391
pPur-ALT1
6622 bp
PacI 1718
mCMV promoter/enh
EcoRI 2015
EcoRI 4507
Swa I 4490
human ALT1
SV40 polyA
BamHI 3756
Sal I 3519

[図18]

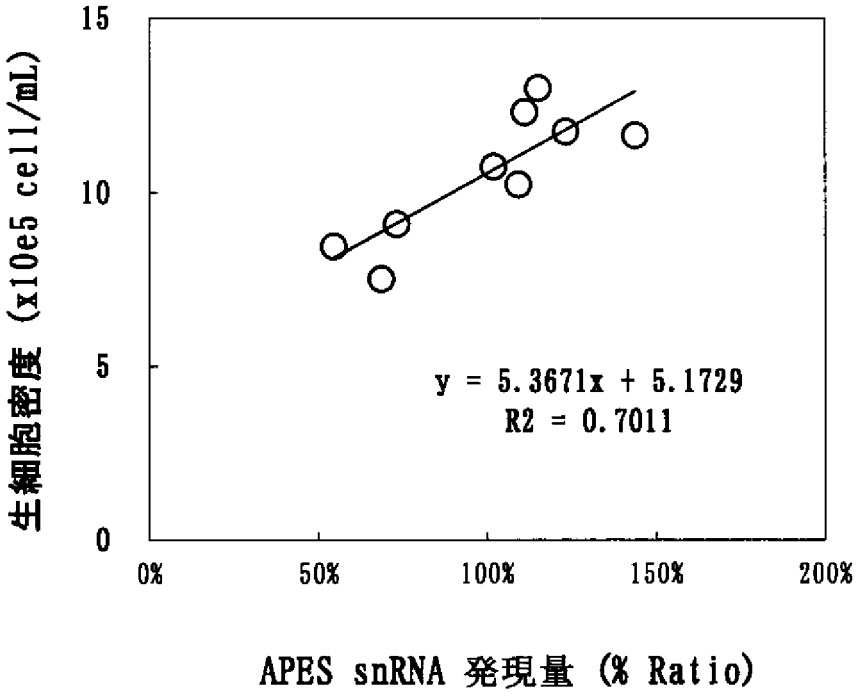
MAb1産生株	細胞最高到達密度 (x10e6 cells/mL)	抗体産生量 (g/L/14日)
APES165 強発現株	11.5 ±1.7	4.4±0.6
ALT1 強発現株	8.9 ±1.8	4.0±0.6
親株	4.1	3.4

シェーカー流加培養 (n=3)

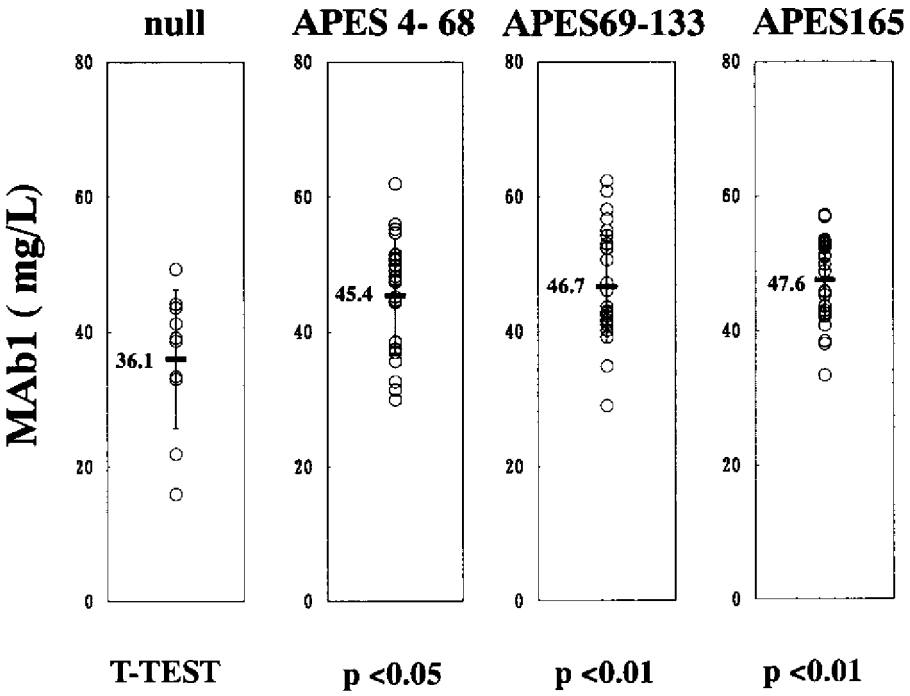
[図19]



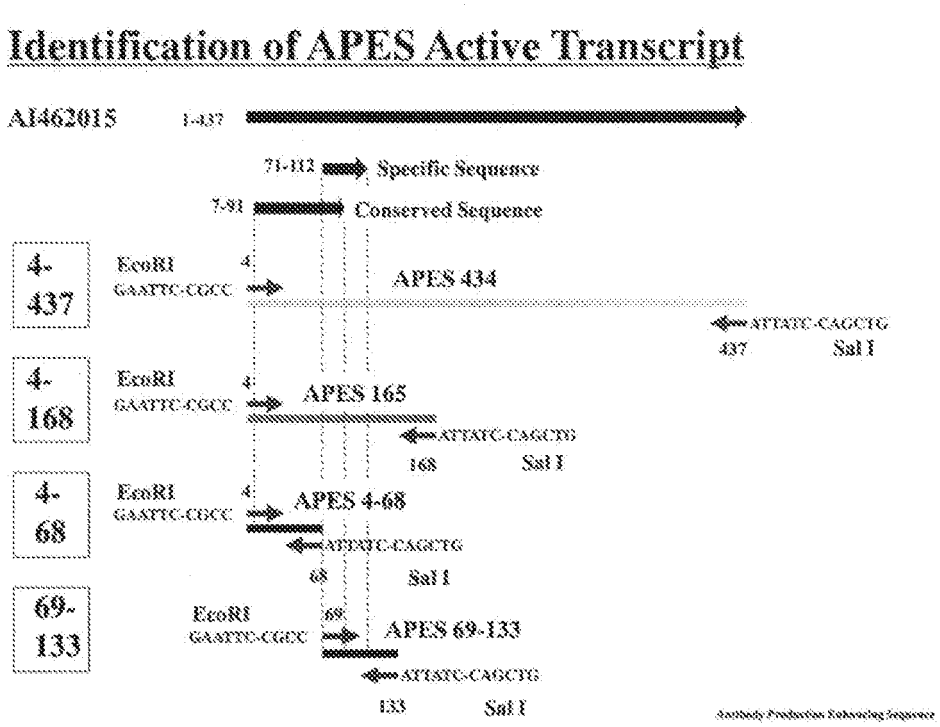
[図20]


15
10
5
0
生細胞密度 (x10e5 cell/mL)
y = 5.3671x + 5.1729
R2 = 0.7011
0%
50%
100%
150%
200%
APES snRNA 発現量 (% Ratio)

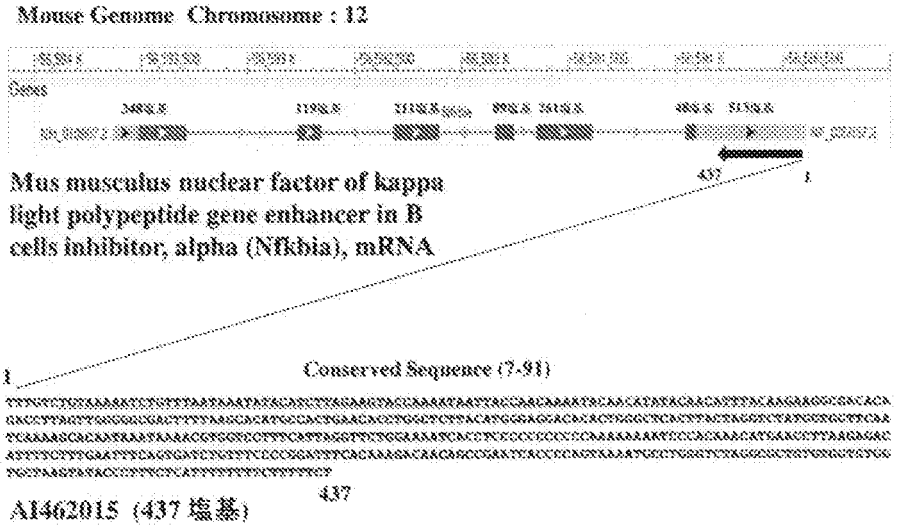
[図21]


null
APES 4- 68
APES69-133
APES165
MAb1 (mg/L)
80
60
40
20
0
36.1
45.4
46.7
47.6
T-TEST
p <0.05
p <0.01
p <0.01

[図22]


Identification of APES Active Transcript
AI462015
1-437
71-112
Specific Sequence
7-91
Conserved Sequence
4-437
EcoRI
GAATTC-CGCC
4
APES 434
ATTATC-CAGCTG
437
Sal I
4-168
EcoRI
GAATTC-CGCC
4
APES 165
ATTATC-CAGCTG
168
Sal I
4-68
EcoRI
GAATTC-CGCC
4
APES 4-68
ATTATC-CAGCTG
68
Sal I
69-133
EcoRI
GAATTC-CGCC
69
APES 69-133
ATTATC-CAGCTG
133
Sal I

[図23]


Mouse Genome Chromosome : 12
Gene
Mus musculus nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, alpha (Nfkbia), mRNA
437
1
1
Conserved Sequence (7-91)
437
AI462015 (437 塩基)

[図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)

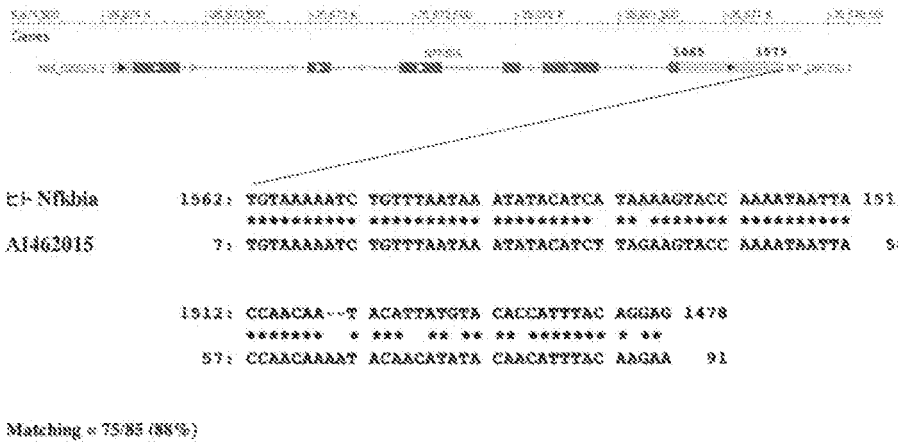
Query 242    CTGGAAAATCACCTooooooooooaaaaaaaTCCCACAAACATGAACCTTAAGAGACA 301    (AI462015)
             ||||||| |||||||| |||| | ||| || ||| | |||| |||||
Sbjct 385443 CTGGAAAACCACCTCCC----------AAACCTCACGAATGTGAGCTTTAAAAGACA 385396 (CHO-K1 genome)

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

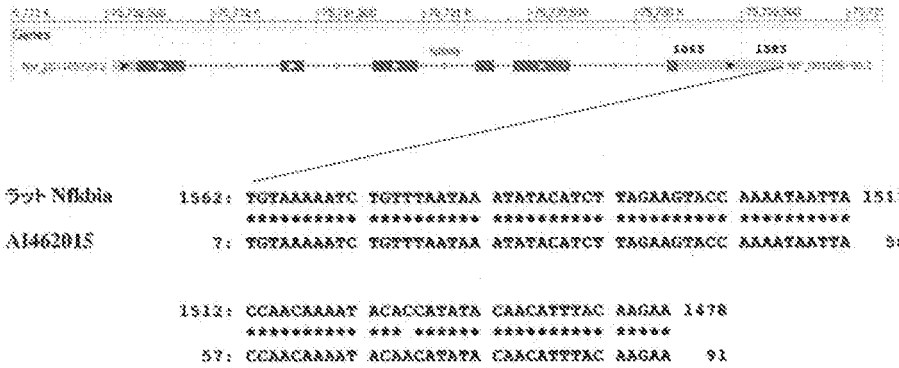
Query 358    TCACCCAGTAAAATGCCTGGGTCTAGGCGCTGTGTGGTGTGGTGCTAAGTATA-CCCTT 416    (AI462015)
             ||||| |||||| |||| |||| |||| |||| |||||||| ||||||||| |||||
Sbjct 385339 TCACCAGAGTAAGATGCTTGGGGCAGGCTGTGTGCAGTGTGGTGGTAAGTATATCCCTT 385280 (CHO-K1 genome)

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

[図25a]

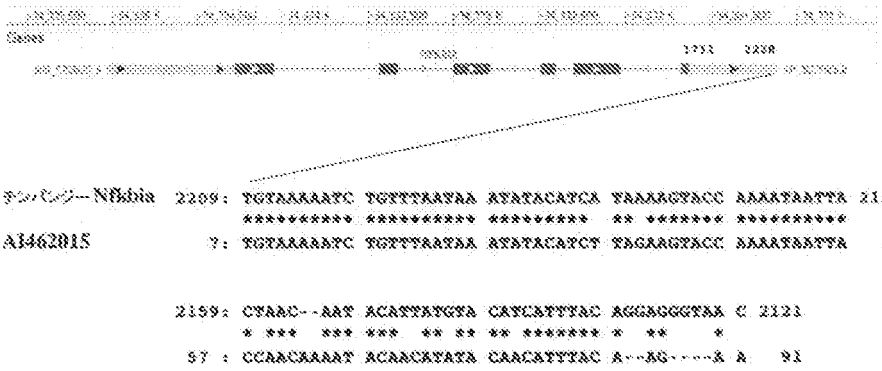


[図25b]



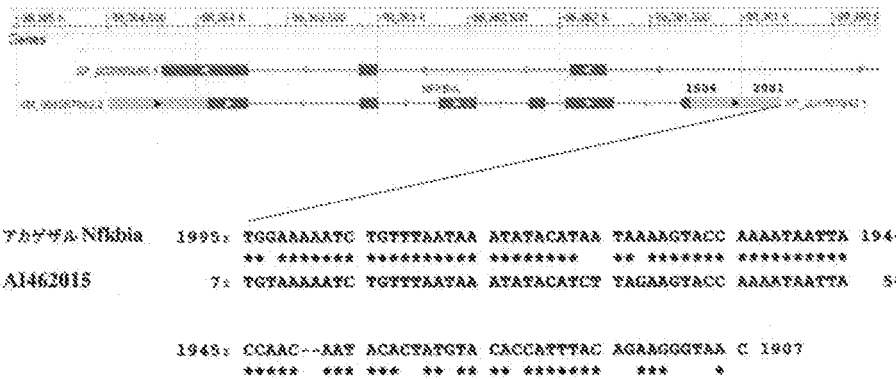
Matching = 84/85 (99%)

[図25c]



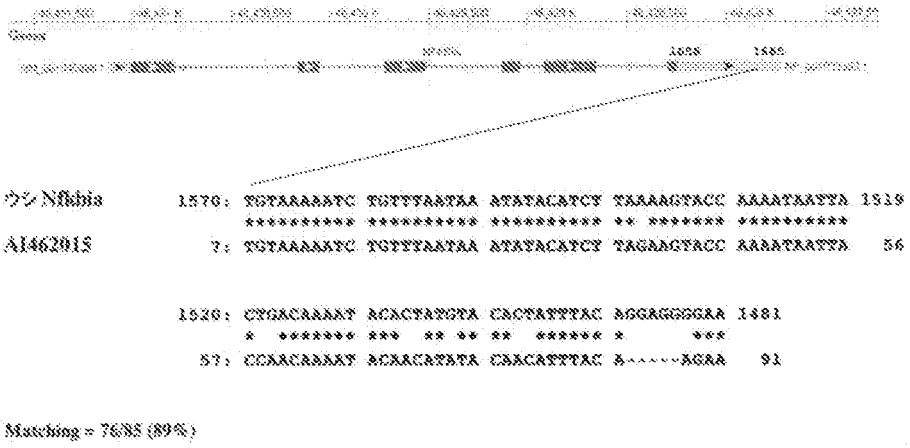
Matching = 75/85 (88%)

[図25d]



Matching = 74/85 (87%)

[図25e]



Matching = 76/85 (89%)