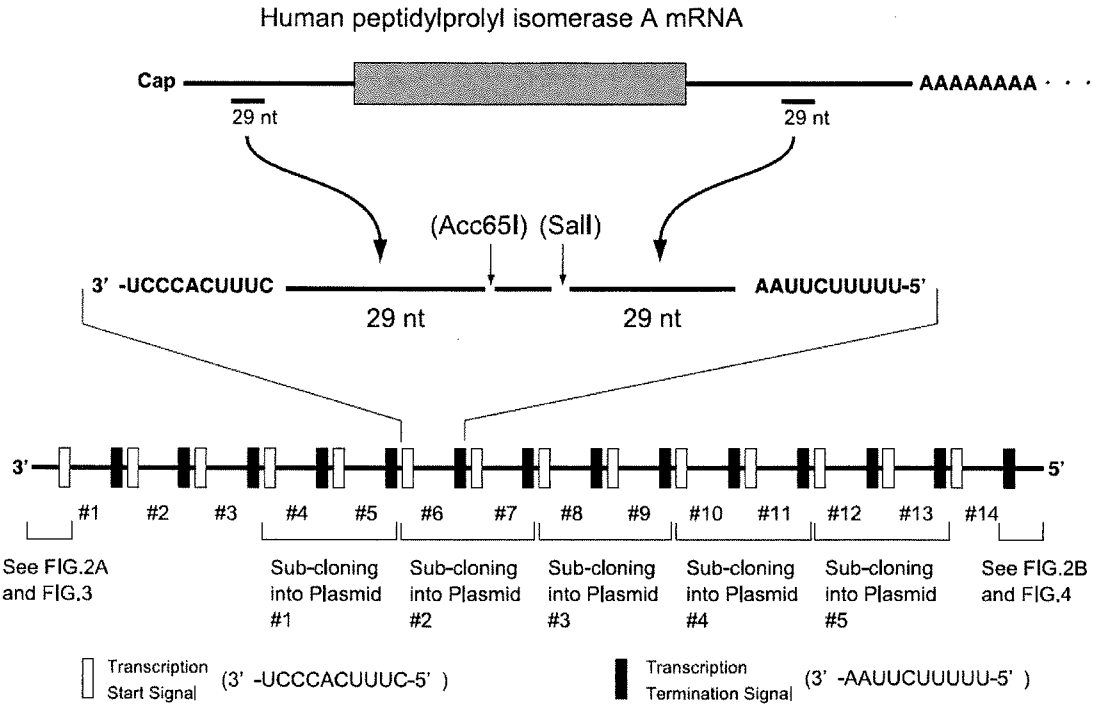
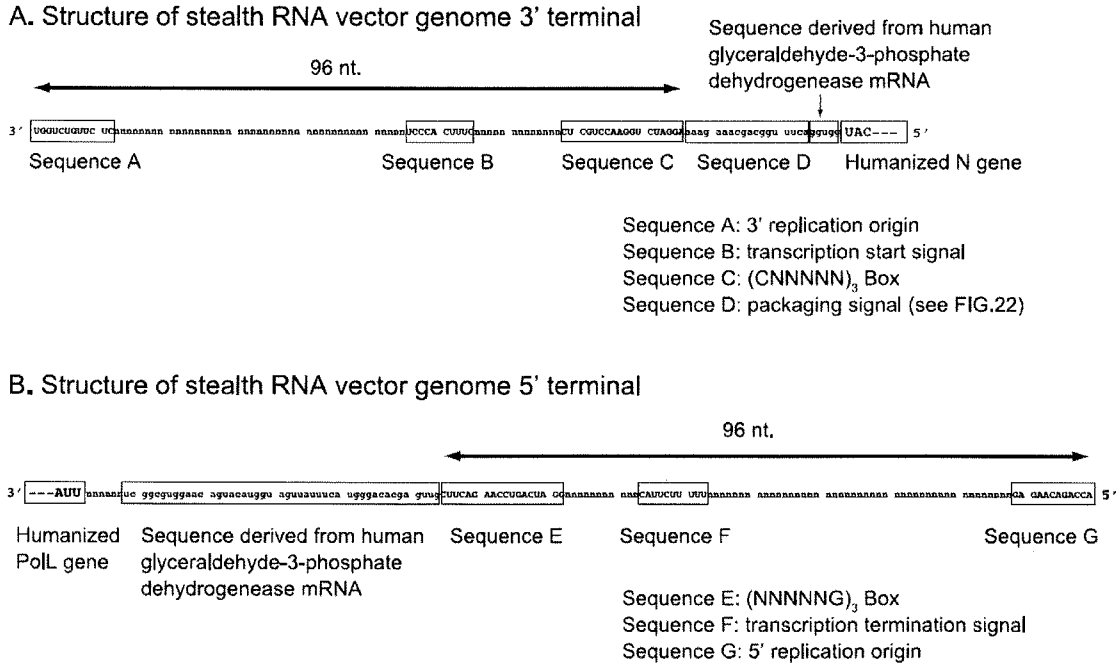
[FIG. 1]

[FIG.1]



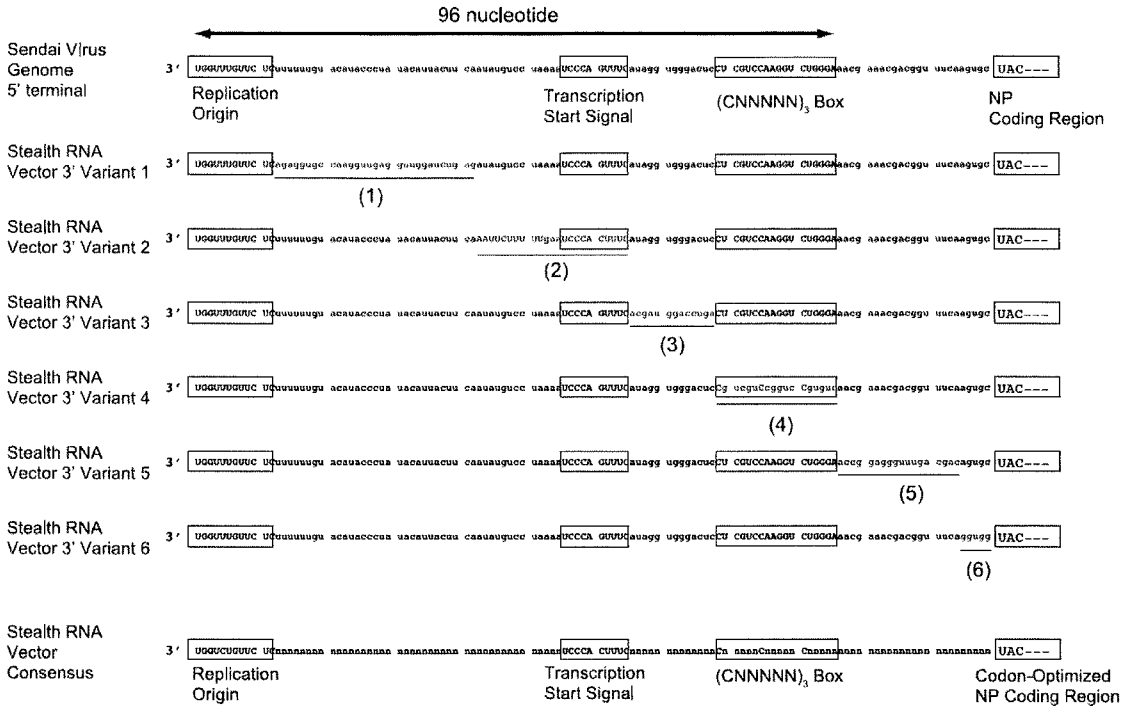
[FIG. 2]

[FIG.2]



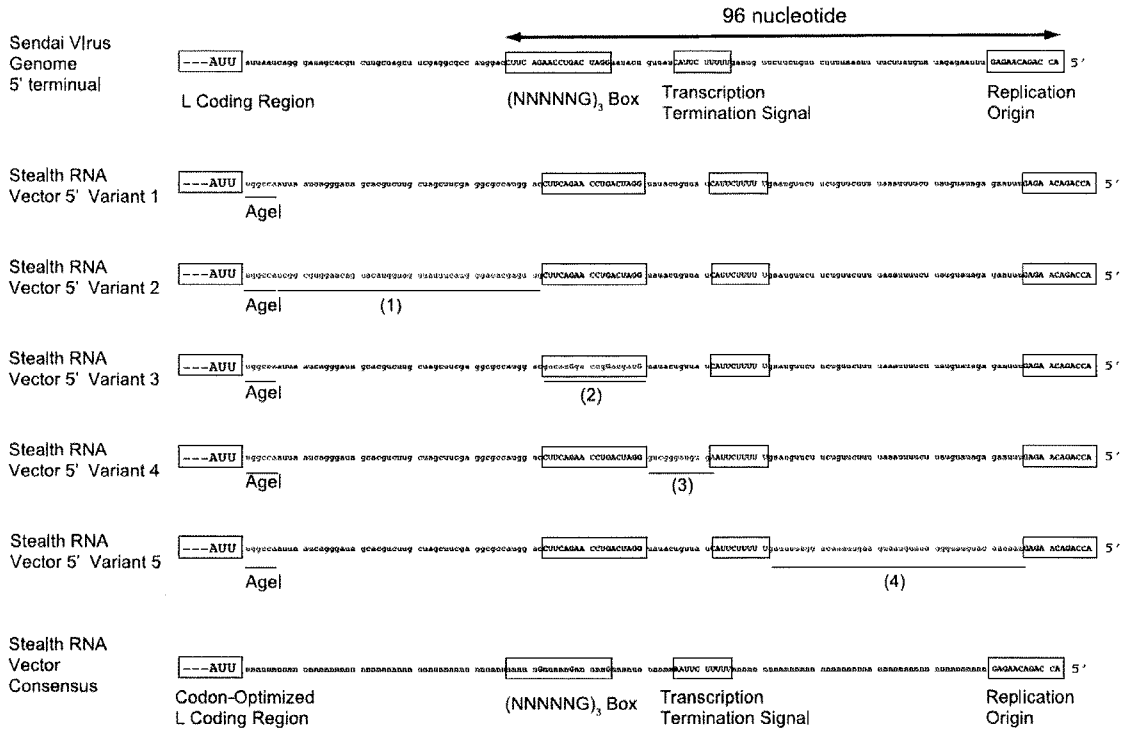
[FIG. 3]

[FIG.3] Structure of stealth RNA vector genome 3' terminal



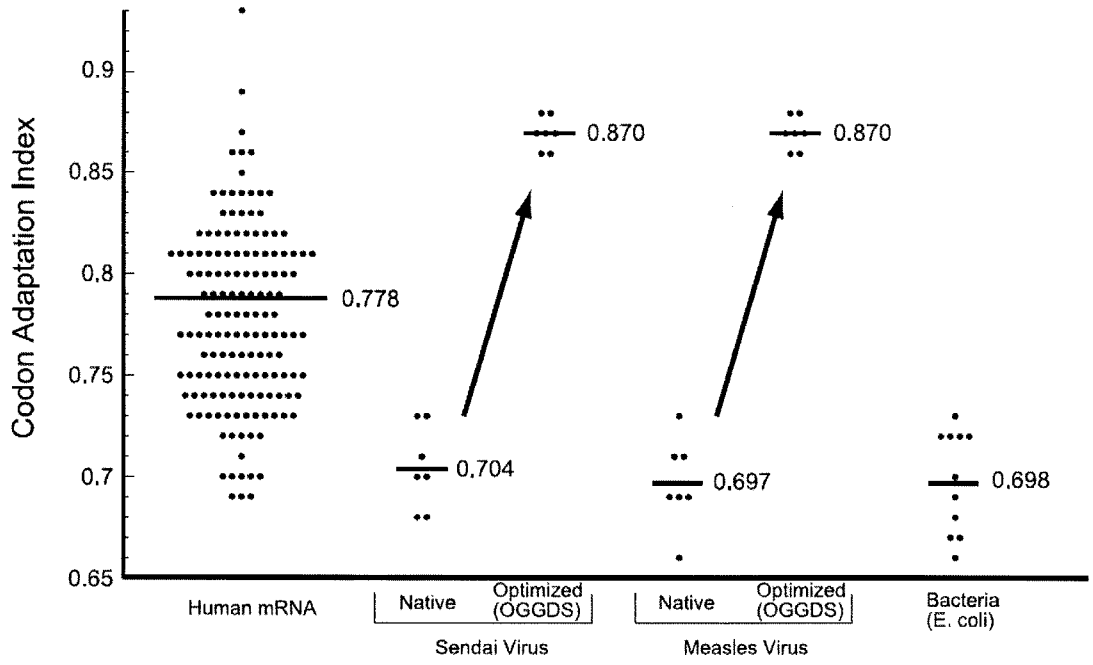
[FIG. 4]

[FIG.4] Structure of stealth RNA vector genome 5 ' terminal



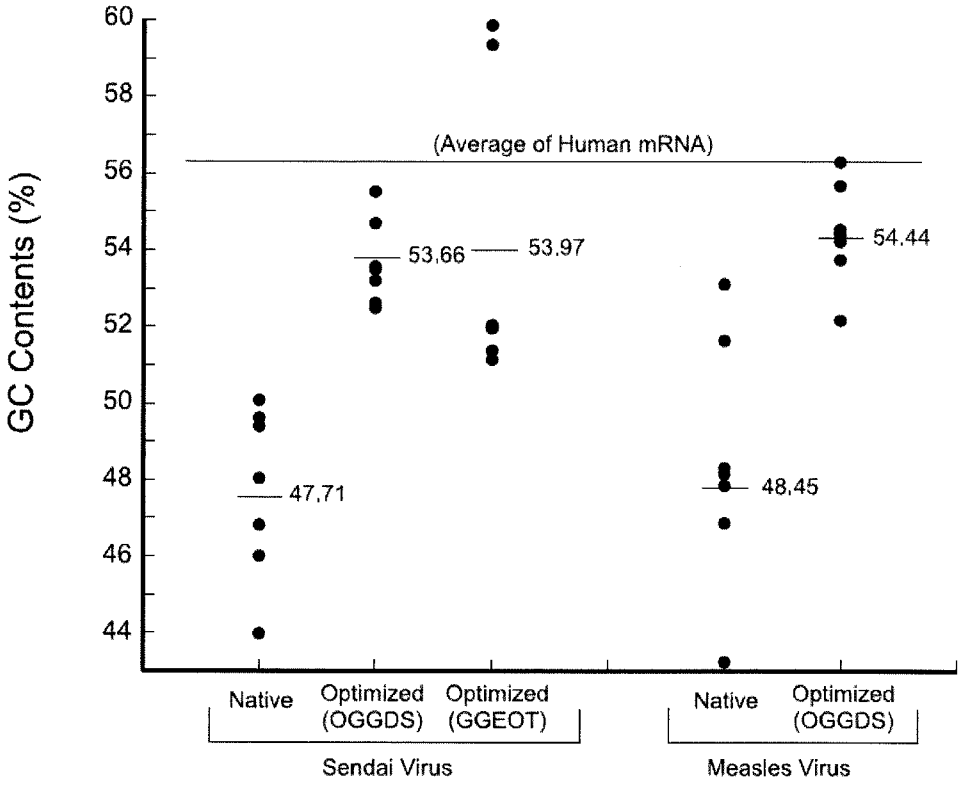
[FIG. 5]

[FIG.5]


Codon Adaptation Index
0.9
0.85
0.8
0.75
0.7
0.65
0.778
0.704
0.870
0.697
0.870
0.698
Human mRNA
Native
Optimized (OGGDS)
Sendai Virus
Native
Optimized (OGGDS)
Measles Virus
Bacteria (E. coli)

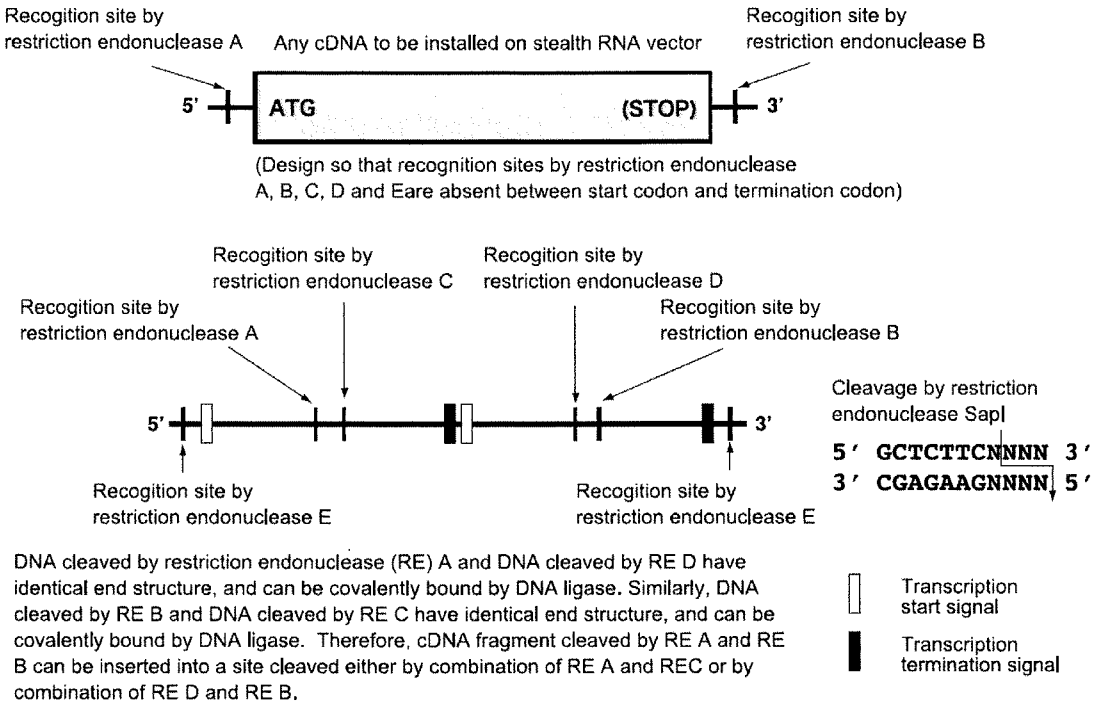
[FIG.6]

[FIG.6]


GC Contents (%)
60
58
56
54
52
50
48
46
44
(Average of Human mRNA)
53.66
53.97
54.44
47.71
48.45
Native
Optimized (OGGDS)
Optimized (GGEOT)
Native
Optimized (OGGDS)
Sendai Virus
Measles Virus

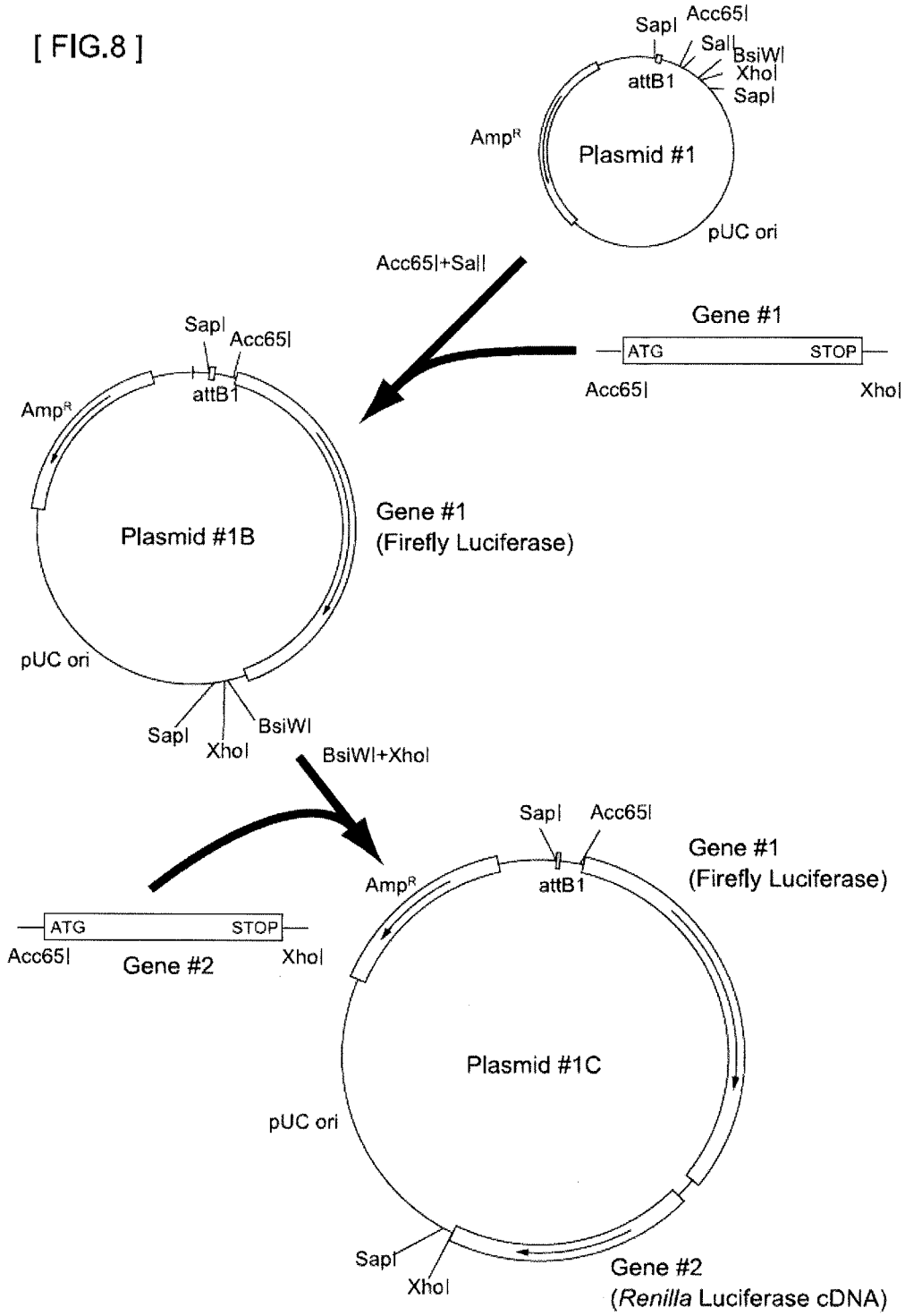
[FIG. 7]

[FIG.7]



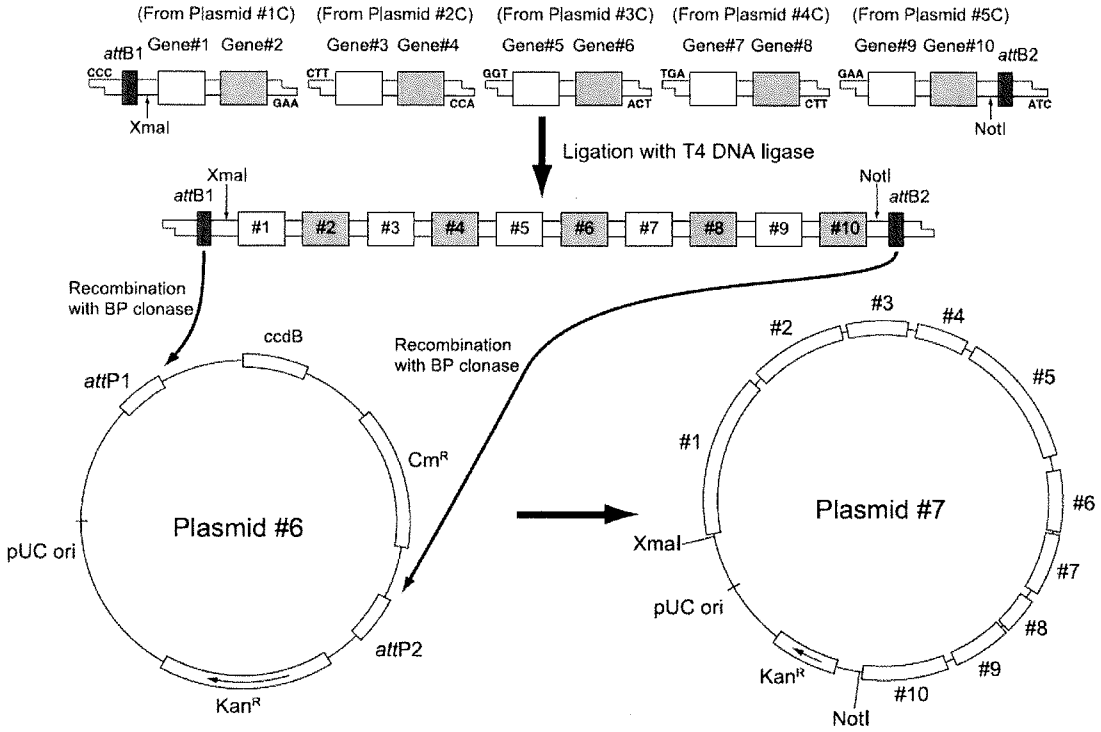
DNA cleaved by restriction endonuclease (RE) A and DNA cleaved by RE D have identical end structure, and can be covalently bound by DNA ligase. Similarly, DNA cleaved by RE B and DNA cleaved by RE C have identical end structure, and can be covalently bound by DNA ligase. Therefore, cDNA fragment cleaved by RE A and RE B can be inserted into a site cleaved either by combination of RE A and REC or by combination of RE D and RE B.

[FIG. 8]

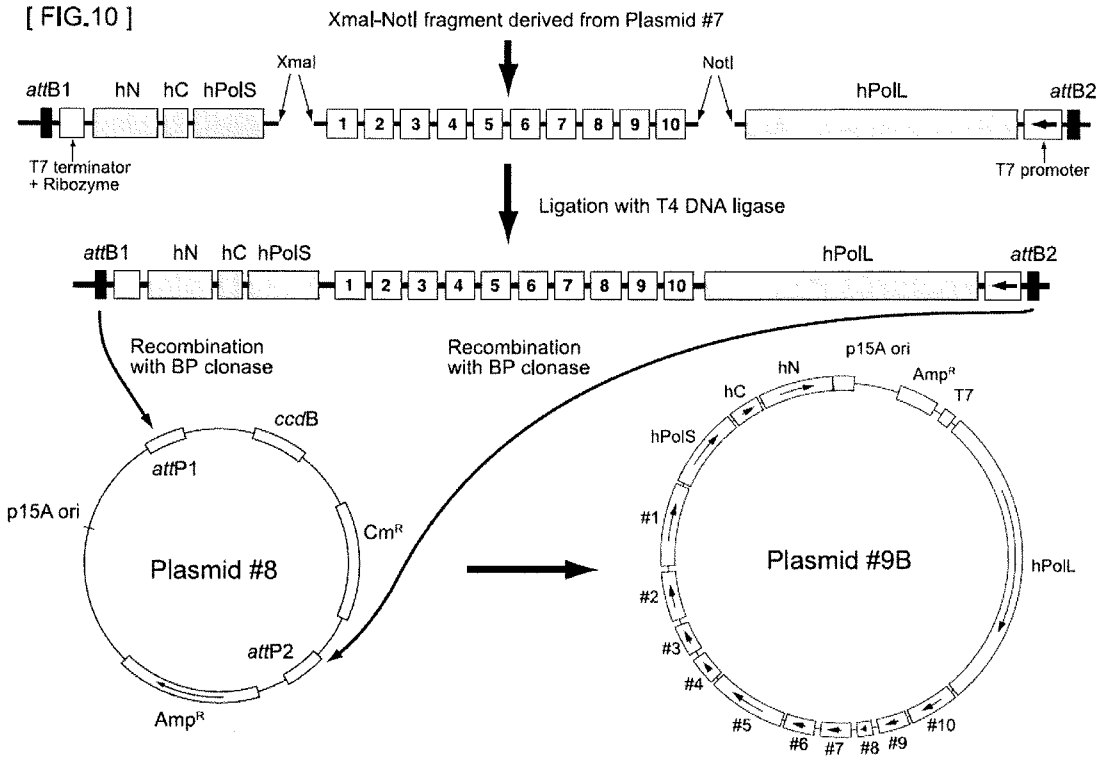

[FIG.8]
SapI
Acc65I
SalI
BsiWI
XhoI
SapI
attB1
AmpR
Plasmid #1
pUC ori
Acc65I+SalI
Gene #1
ATG
STOP
Acc65I
XhoI
SapI
Acc65I
attB1
AmpR
Plasmid #1B
Gene #1
(Firefly Luciferase)
pUC ori
SapI
XhoI
BsiWI
BsiWI+XhoI
SapI
Acc65I
Gene #1
(Firefly Luciferase)
AmpR
attB1
ATG
STOP
Acc65I
Gene #2
XhoI
Plasmid #1C
pUC ori
SapI
XhoI
Gene #2
(Renilla Luciferase cDNA)

[FIG. 9]

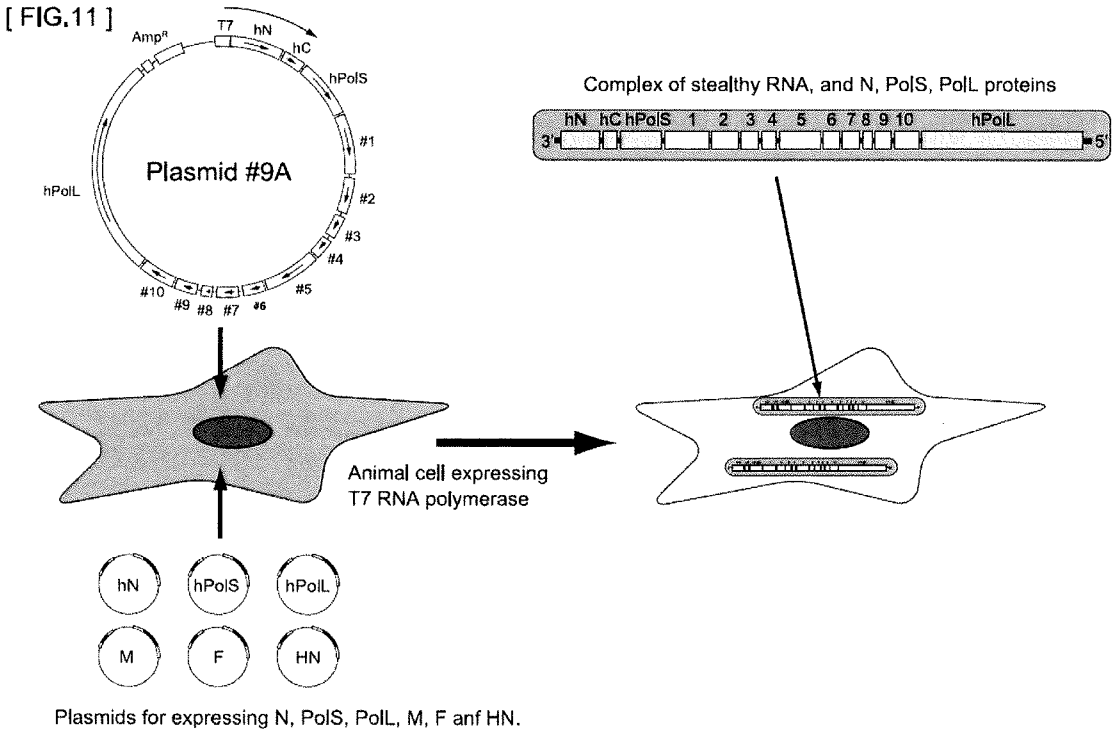
[FIG.9]


(From Plasmid #1C)
(From Plasmid #2C)
(From Plasmid #3C)
(From Plasmid #4C)
(From Plasmid #5C)
attB1
Gene#1
Gene#2
Gene#3
Gene#4
Gene#5
Gene#6
Gene#7
Gene#8
Gene#9
Gene#10
attB2
CCC
GAA
CTT
CCA
GGT
ACT
TGA
CTT
GAA
ATC
XmaI
NotI
Ligation with T4 DNA ligase
XmaI
attB1
NotI
attB2
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
Recombination with BP clonase
ccdB
attP1
Cm^R
Plasmid #6
pUC ori
attP2
Kan^R
Recombination with BP clonase
#3
#2
#4
#5
#1
#6
Plasmid #7
XmaI
#7
pUC ori
#8
Kan^R
#9
#10
NotI

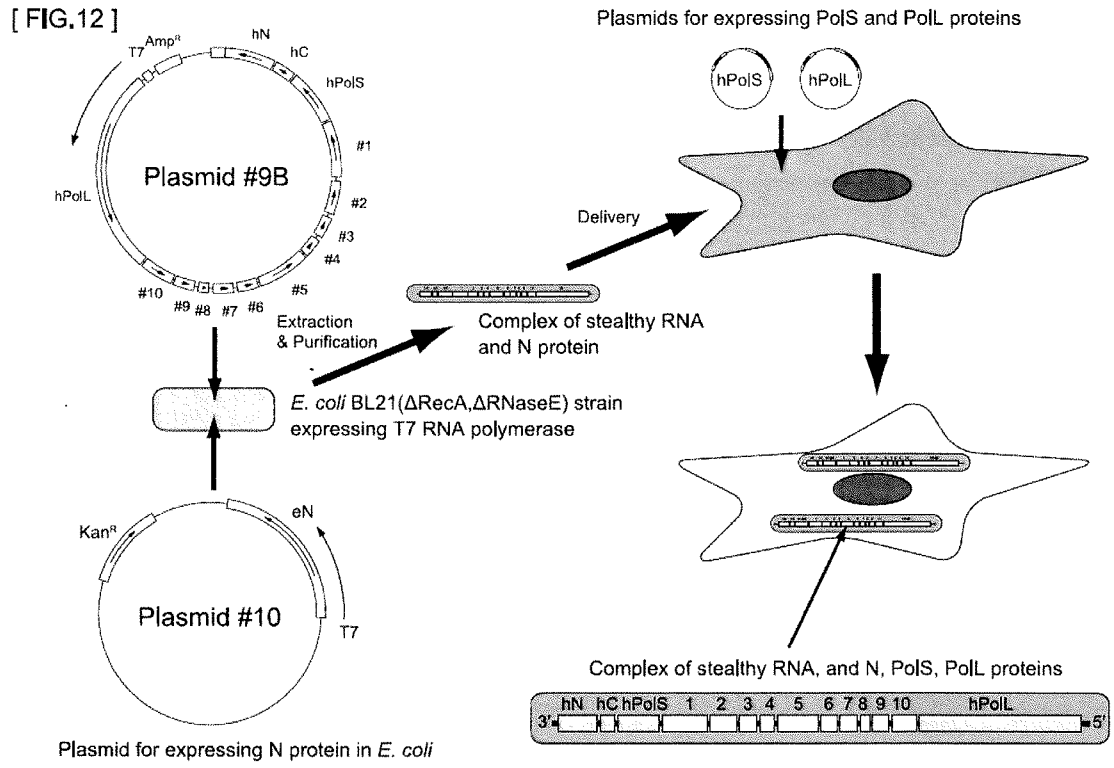
[FIG. 10]


[FIG.10]
XmaI-NotI fragment derived from Plasmid #7
XmaI
NotI
attB1
hN
hC
hPolS
1 2 3 4 5 6 7 8 9 10
hPolL
attB2
T7 terminator
+ Ribozyme
T7 promoter
Ligation with T4 DNA ligase
Recombination
with BP clonase
Recombination
with BP clonase
ccdB
attP1
p15A ori
CmR
Plasmid #8
attP2
AmpR
p15A ori
hN
AmpR
T7
hC
hPolS
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
Plasmid #9B
hPolL

[FIG. 11]

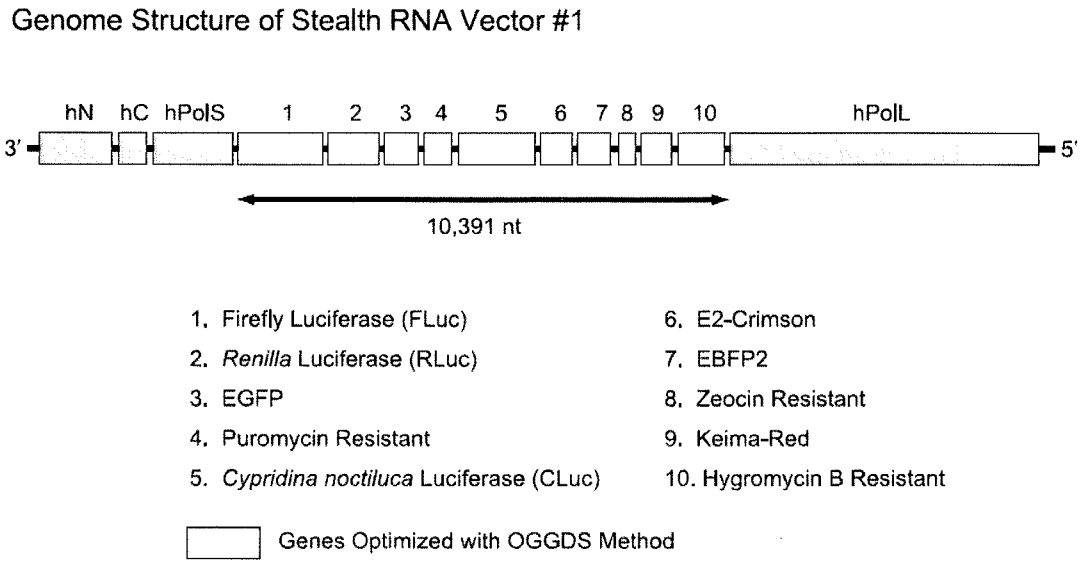

[FIG.11]
AmpR
T7
hN
hC
hPolS
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
Plasmid #9A
hPolL
Complex of stealthy RNA, and N, PolS, PolL proteins
hN hC hPolS 1 2 3 4 5 6 7 8 9 10 hPolL
3'
5'
Animal cell expressing
T7 RNA polymerase
hN
hPolS
hPolL
M
F
HN
Plasmids for expressing N, PolS, PolL, M, F anf HN.

[FIG. 12]



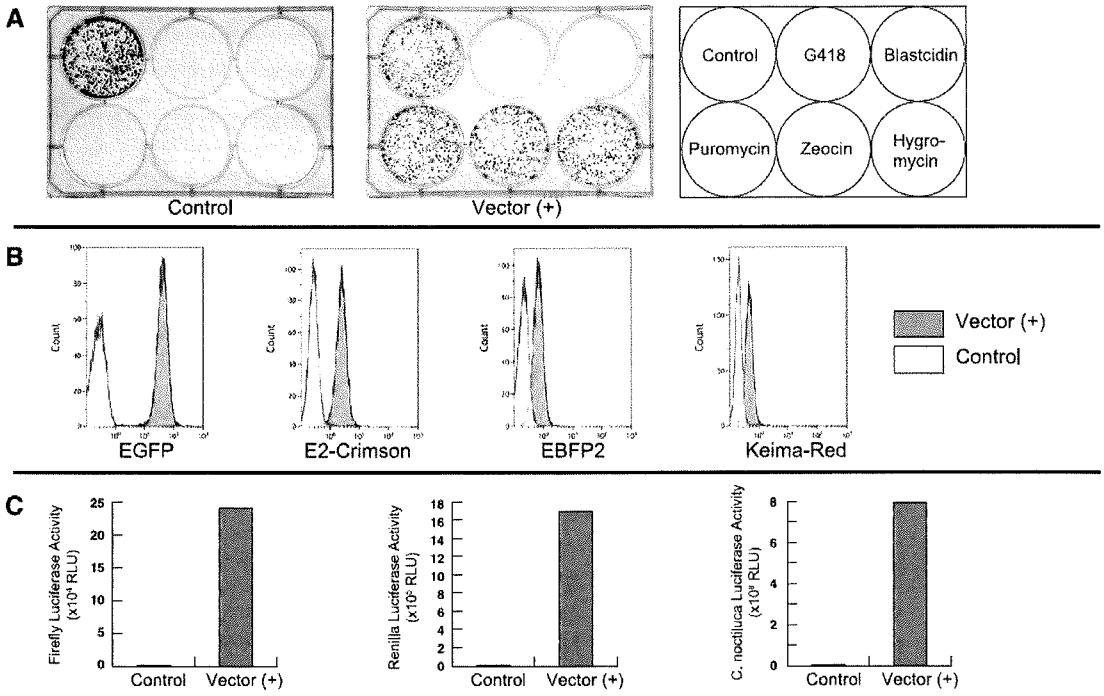
[FIG. 13]

[FIG.13]



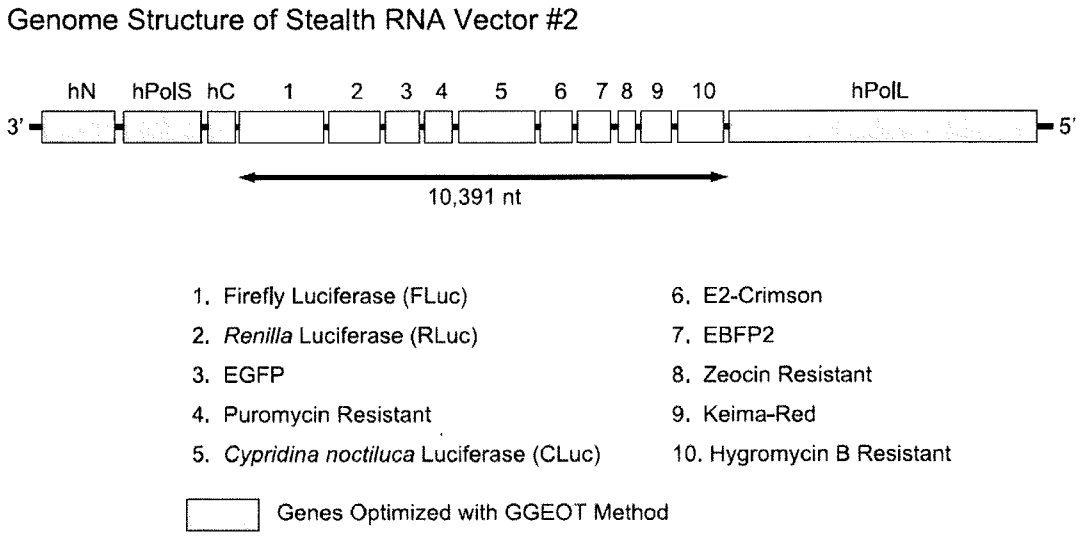
[FIG. 14]

[FIG.14]



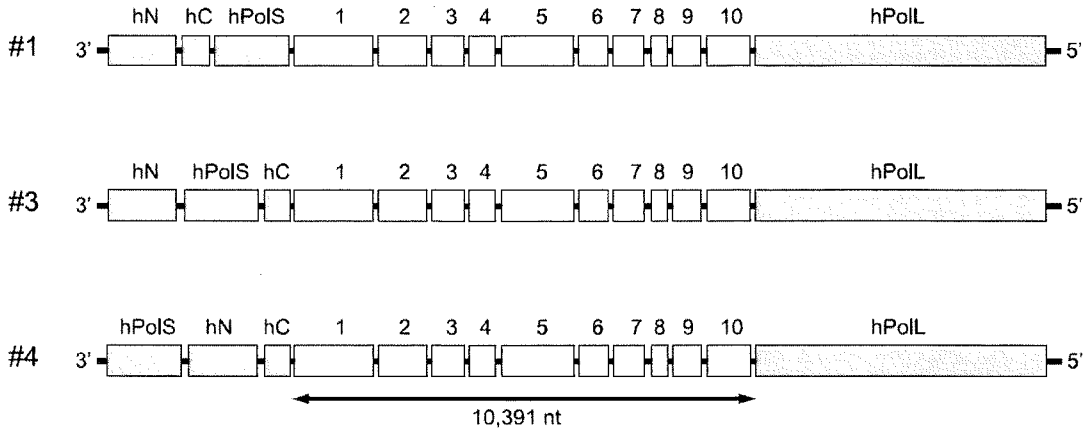
[FIG. 15]

[FIG.15]



[FIG. 16]

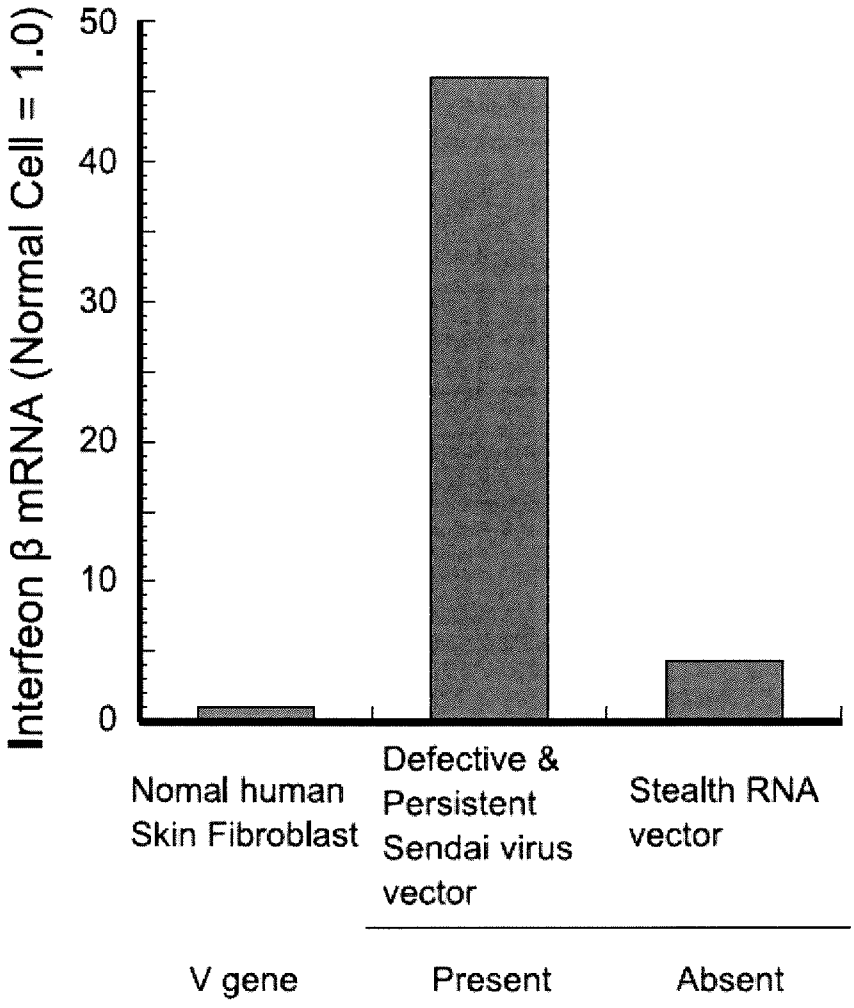
[FIG.16]



1. Firefly Luciferase (FLuc)
2. *Renilla* Luciferase (RLuc)
3. EGFP
4. Puromycin Resistant
5. *Cypridina noctiluca* Luciferase (CLuc)
6. E2-Crimson
7. EBFP2
8. Zeocin Resistant
9. Keima-Red
10. Hygromycin B Resistant

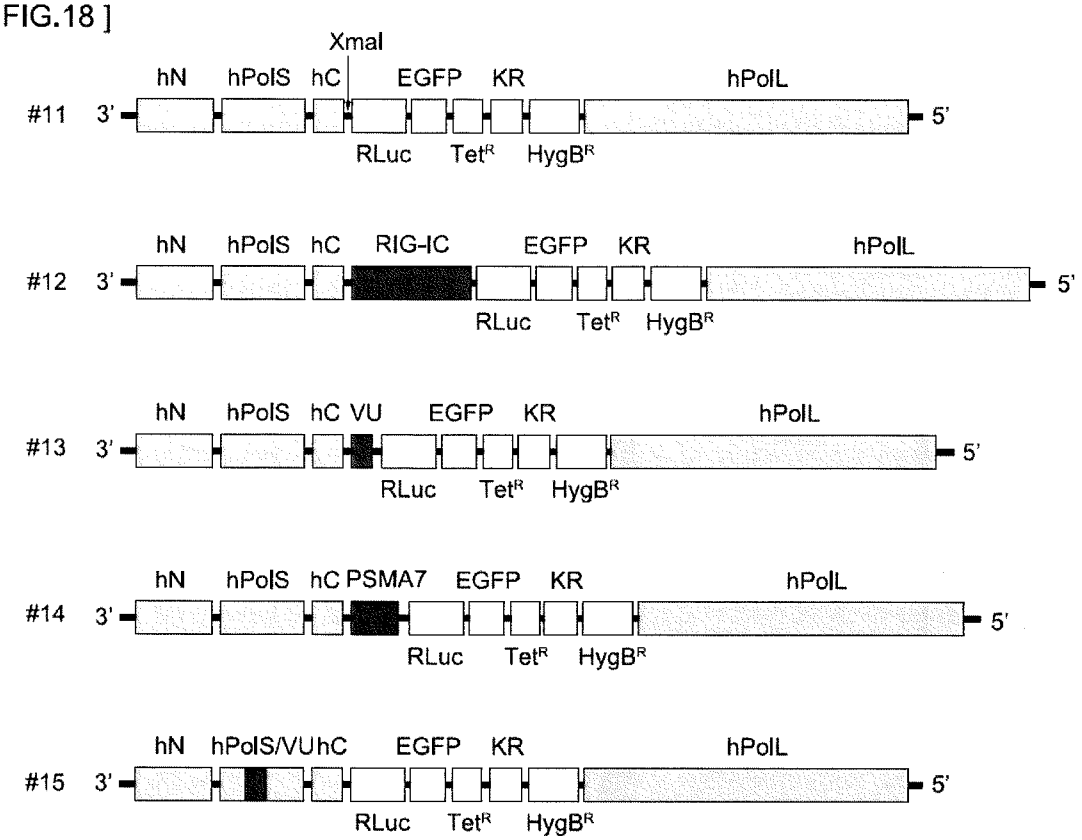
[FIG. 17]

[FIG.17]


Interfeon β mRNA (Normal Cell = 1.0)
50
40
30
20
10
0
Nomal human Skin Fibroblast
Defective & Persistent Sendai virus vector
Stealth RNA vector
V gene
Present
Absent

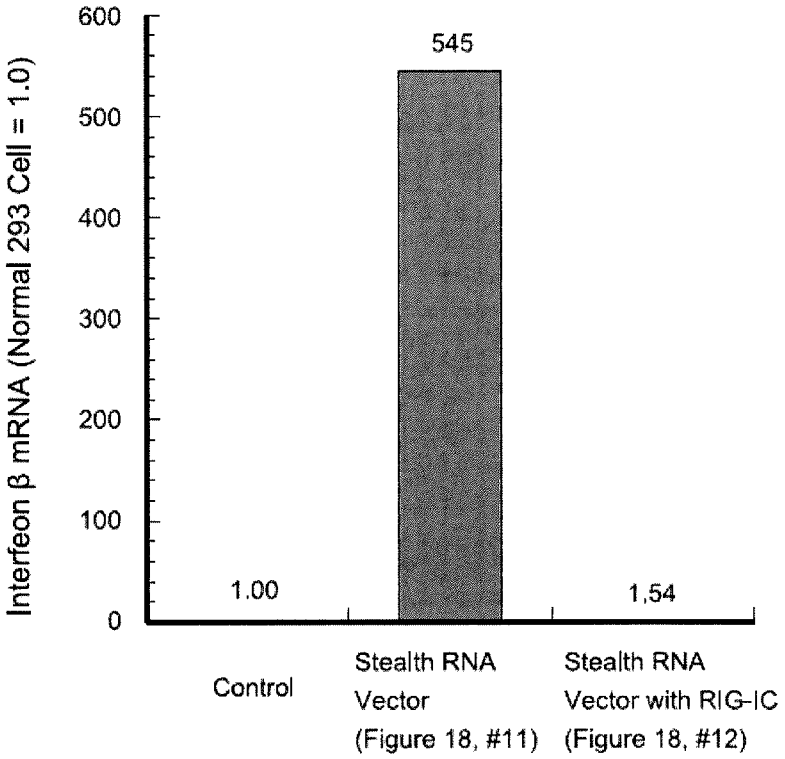
[FIG. 18]

[FIG.18]


XmaI
hN
hPolS
hC
EGFP
KR
hPolL
#11
3'
5'
RLuc
Tet^R
HygB^R
hN
hPolS
hC
RIG-IC
EGFP
KR
hPolL
#12
3'
5'
RLuc
Tet^R
HygB^R
hN
hPolS
hC VU
EGFP
KR
hPolL
#13
3'
5'
RLuc
Tet^R
HygB^R
hN
hPolS
hC PSMA7
EGFP
KR
hPolL
#14
3'
5'
RLuc
Tet^R
HygB^R
hN
hPolS/VUhC
EGFP
KR
hPolL
#15
3'
5'
RLuc
Tet^R
HygB^R

[FIG. 19]

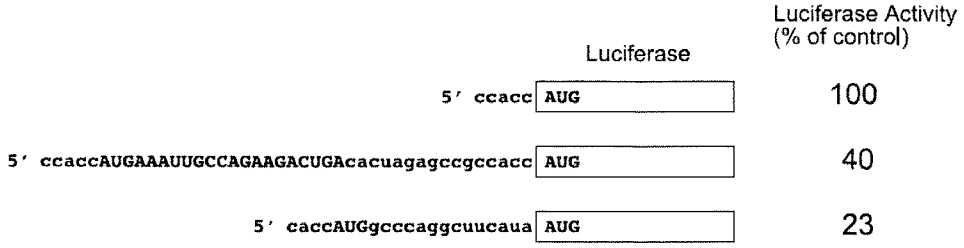
[FIG.19]


Interfeon β mRNA (Normal 293 Cell = 1.0)
600
500
400
300
200
100
0
545
1.00
1,54
Control
Stealth RNA Vector (Figure 18, #11)
Stealth RNA Vector with RIG-IC (Figure 18, #12)

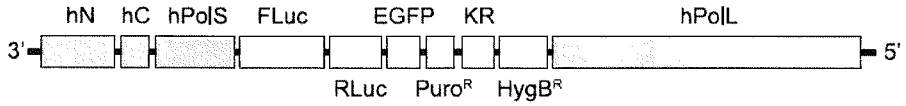
[FIG. 20]

[FIG.20]

A



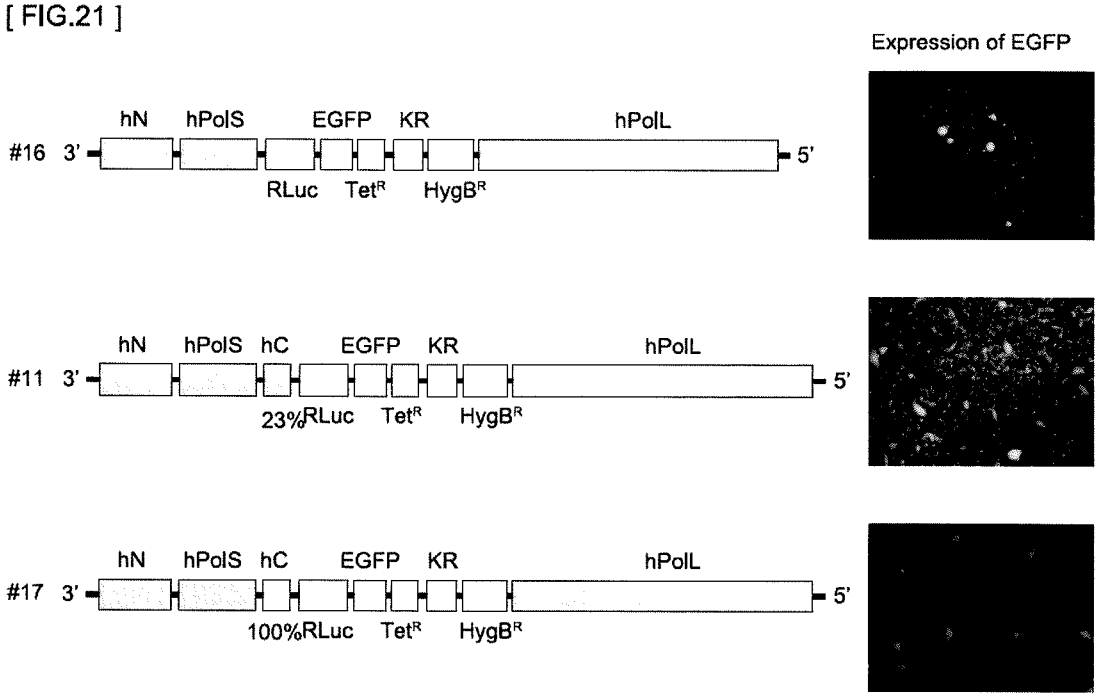
B



	Translation Efficiency		F.Luciferase Activity (Relative value)
	NP	C	
Stealth RNA gene expression system #6	100	100	1.0
Stealth RNA gene expression system #7	100	23	8.8
Stealth RNA gene expression system #8	40	23	79.0

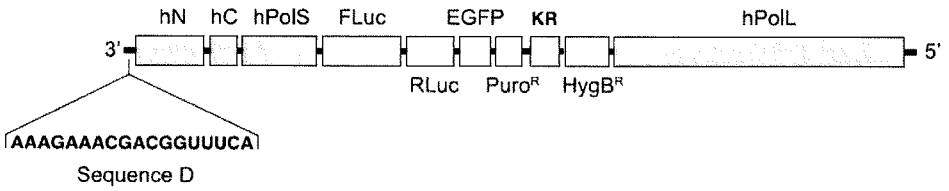
[FIG. 21]

[FIG.21]



[FIG. 22]

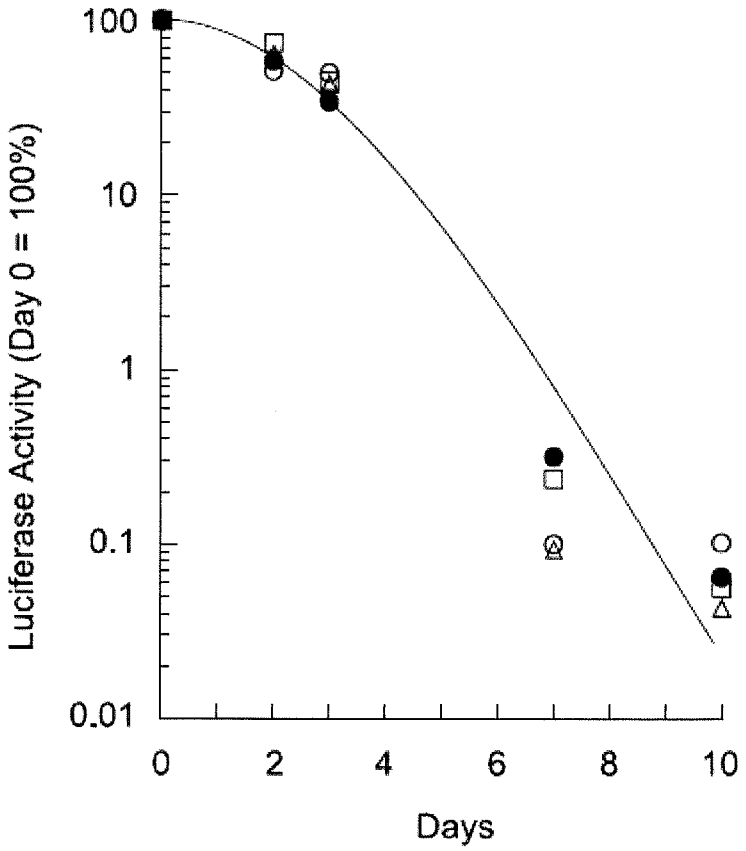
[FIG.22]



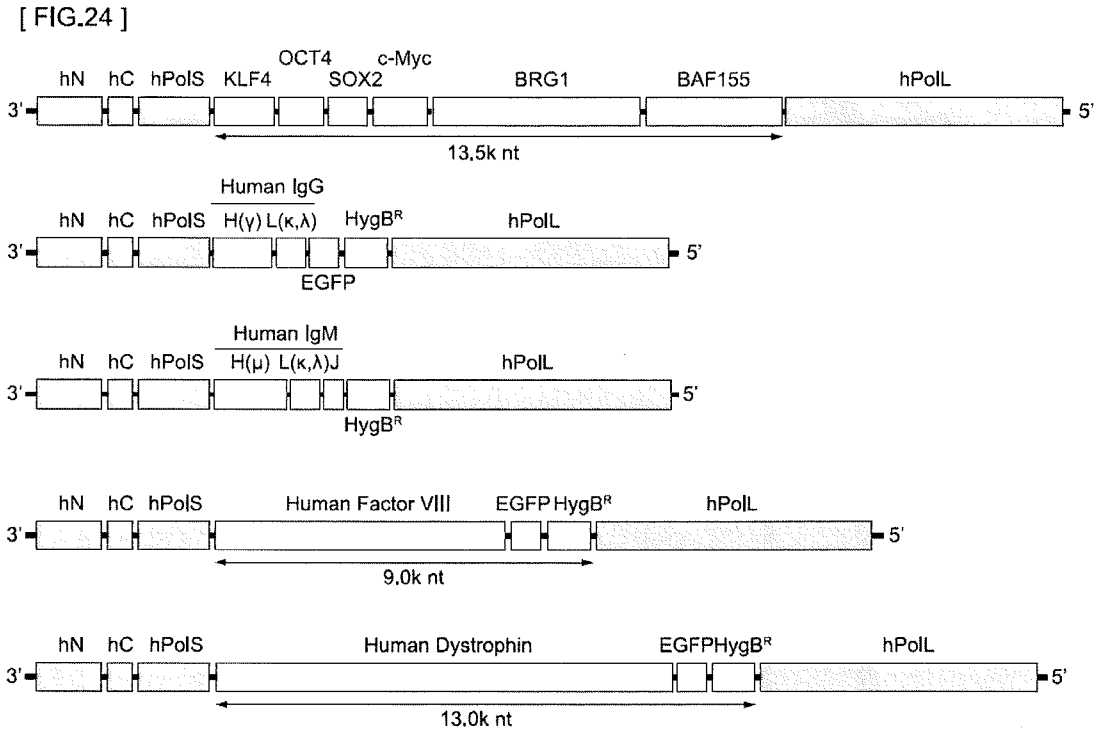
		Expression of EGFP in packaging cells	Number of vector particles in packaging cell supernatant (x 10^4 /mL)
Stealth RNA gene expression system #9	Remove Sequence D From #6	+++	2
Stealth RNA gene expression system #6		+++	210
Stealth RNA gene expression system #10	Remove Sequence D From #7	++++	7
Stealth RNA gene expression system #7		++++	450

[FIG. 23]

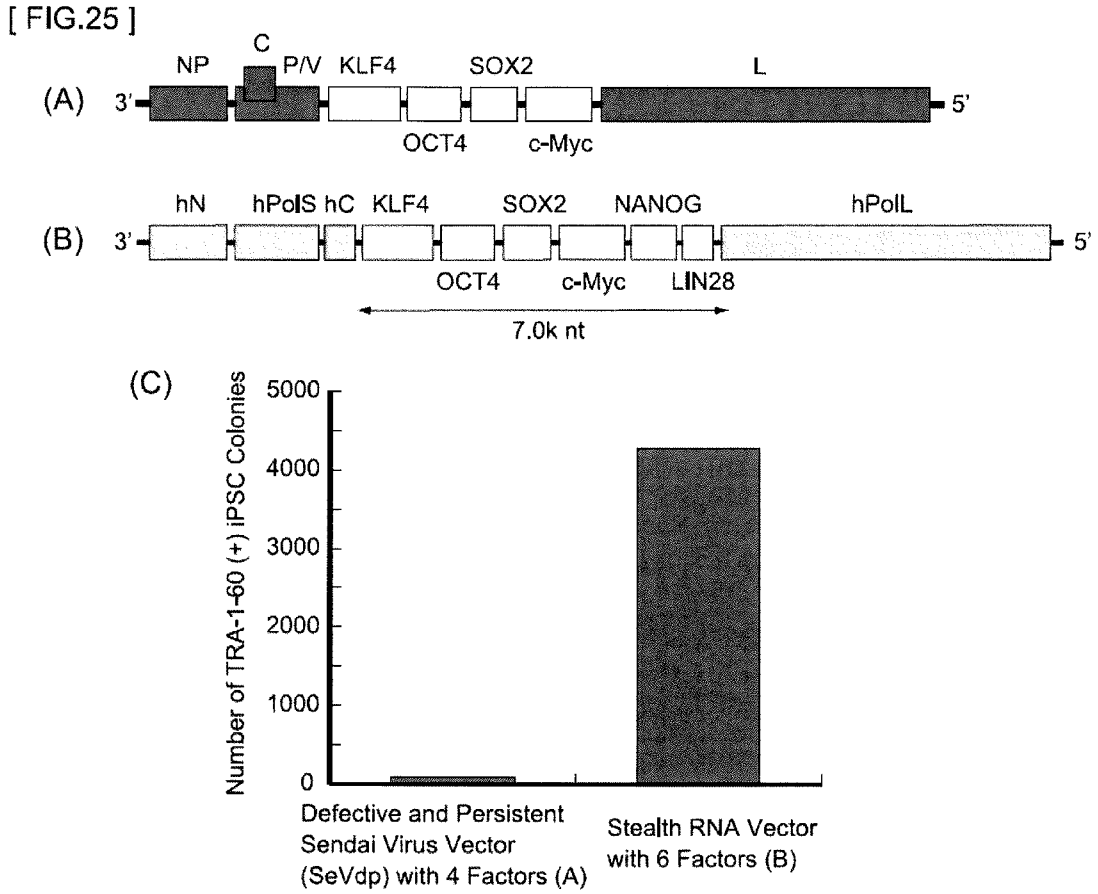
[FIG.23]



[FIG. 24]

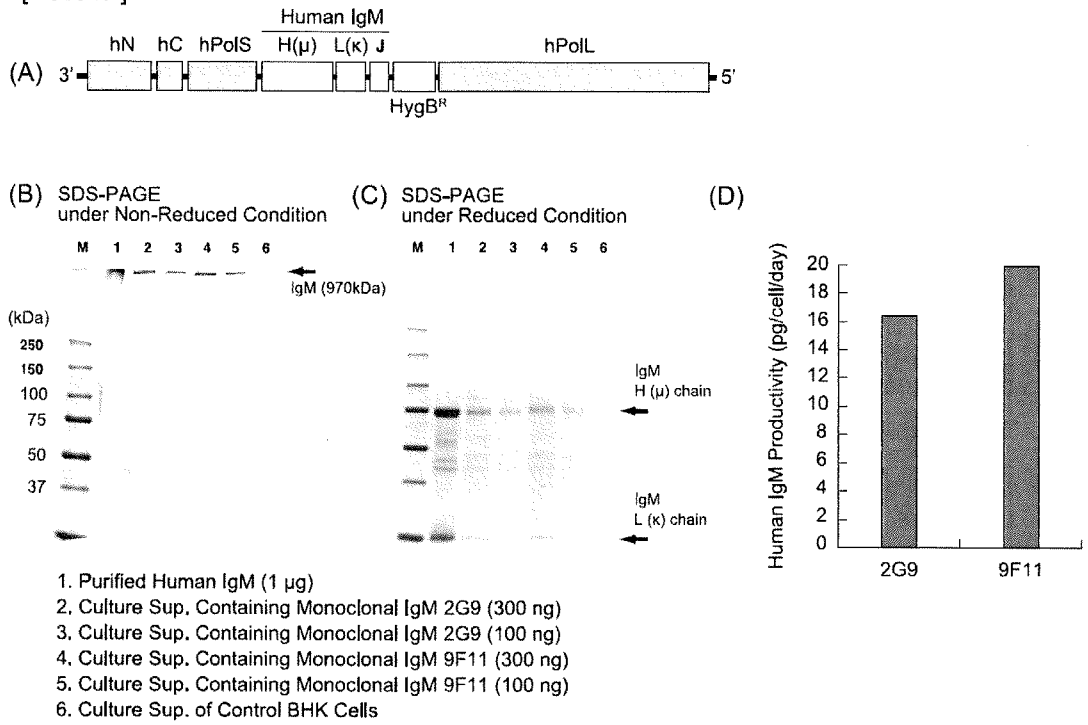

[FIG.24]
hN
hC
hPolS
KLF4
OCT4
c-Myc
SOX2
BRG1
BAF155
hPolL
3'
5'
13.5k nt
Human IgG
H(γ) L(κ,λ)
HygB^R
EGFP
Human IgM
H(μ) L(κ,λ)J
Human Factor VIII
EGFP
9.0k nt
Human Dystrophin
EGFPHygB^R
13.0k nt

[FIG. 25]


[FIG.25]
(A)
3'
NP
C
P/V
KLF4
SOX2
L
5'
OCT4
c-Myc
(B)
3'
hN
hPolS
hC
KLF4
SOX2
NANOG
hPolL
5'
OCT4
c-Myc
LIN28
7.0k nt
(C)
Number of TRA-1-60 (+) iPSC Colonies
5000
4000
3000
2000
1000
0
Defective and Persistent Sendai Virus Vector (SeVdp) with 4 Factors (A)
Stealth RNA Vector with 6 Factors (B)

[FIG. 26]

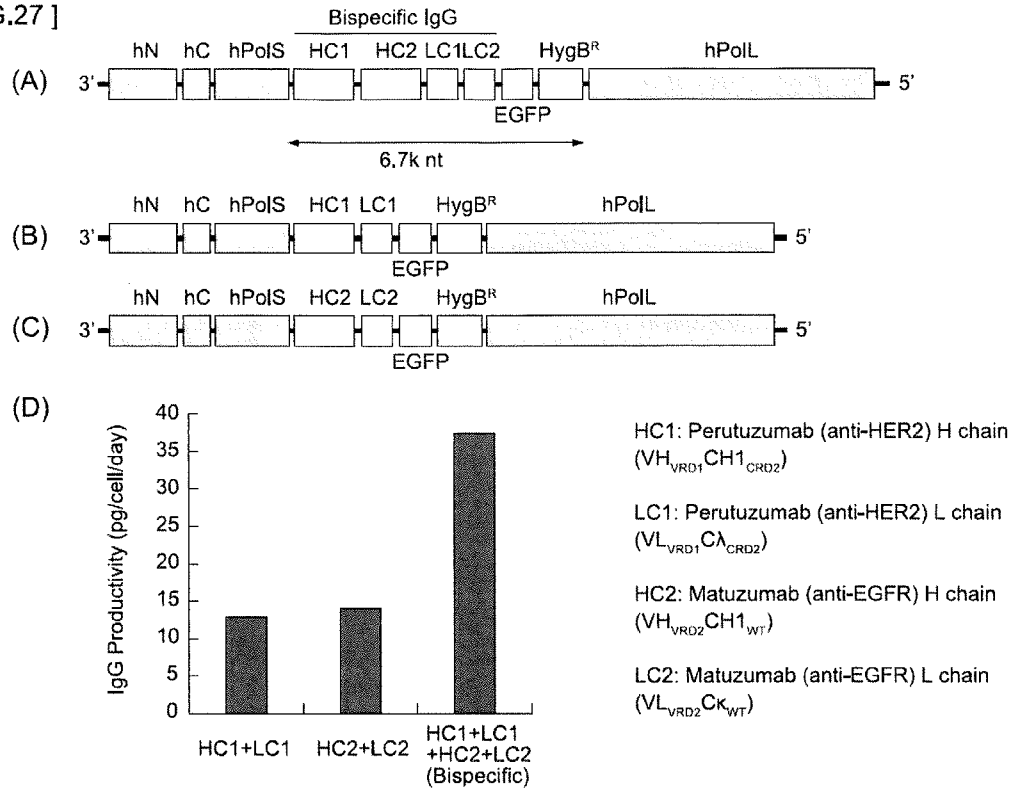
[FIG.26]



1. Purified Human IgM (1 μg)
2. Culture Sup. Containing Monoclonal IgM 2G9 (300 ng)
3. Culture Sup. Containing Monoclonal IgM 2G9 (100 ng)
4. Culture Sup. Containing Monoclonal IgM 9F11 (300 ng)
5. Culture Sup. Containing Monoclonal IgM 9F11 (100 ng)
6. Culture Sup. of Control BHK Cells

[FIG. 27]

[FIG.27]


Bispecific IgG
hN hC hPolS HC1 HC2 LC1LC2 HygB^R hPolL
(A) 3' 5'
EGFP
6.7k nt
hN hC hPolS HC1 LC1 HygB^R hPolL
(B) 3' 5'
EGFP
hN hC hPolS HC2 LC2 HygB^R hPolL
(C) 3' 5'
EGFP
(D)
IgG Productivity (pg/cell/day)
40
35
30
25
20
15
10
5
0
HC1+LC1
HC2+LC2
HC1+LC1 +HC2+LC2 (Bispecific)
HC1: Perutuzumab (anti-HER2) H chain ($VH_{VRD1}CH1_{CRD2}$)
LC1: Perutuzumab (anti-HER2) L chain ($VL_{VRD1}C\lambda_{CRD2}$)
HC2: Matuzumab (anti-EGFR) H chain ($VH_{VRD2}CH1_{WT}$)
LC2: Matuzumab (anti-EGFR) L chain ($VL_{VRD2}C\kappa_{WT}$)