Fig. 1

(-) : FuGENE-HD alone

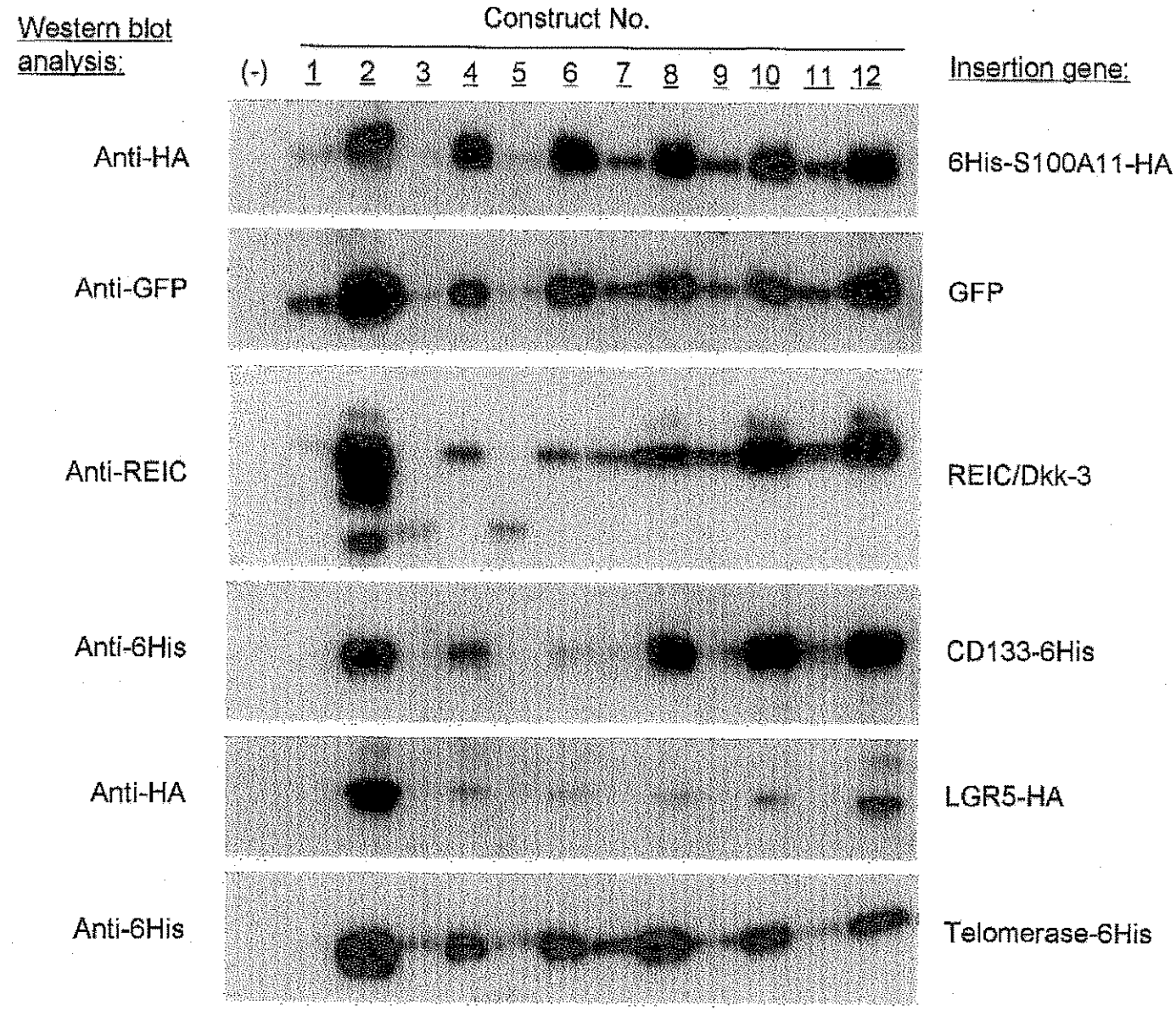


Fig. 2

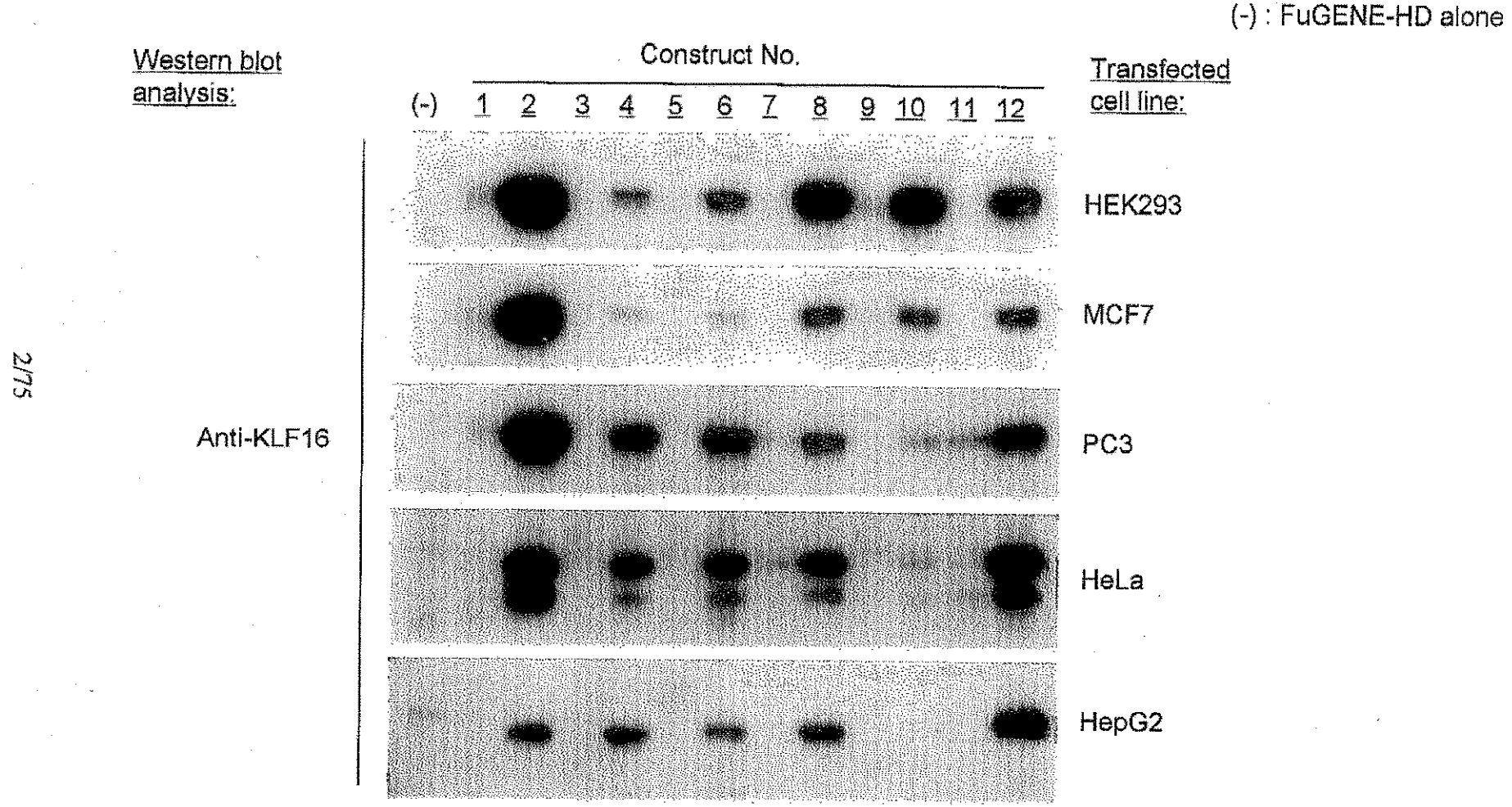

(-) : FuGENE-HD alone
Western blot analysis:
Construct No.
(-) 1 2 3 4 5 6 7 8 9 10 11 12
Transfected cell line:
Anti-KLF16
HEK293
MCF7
PC3
HeLa
HepG2

Fig. 3

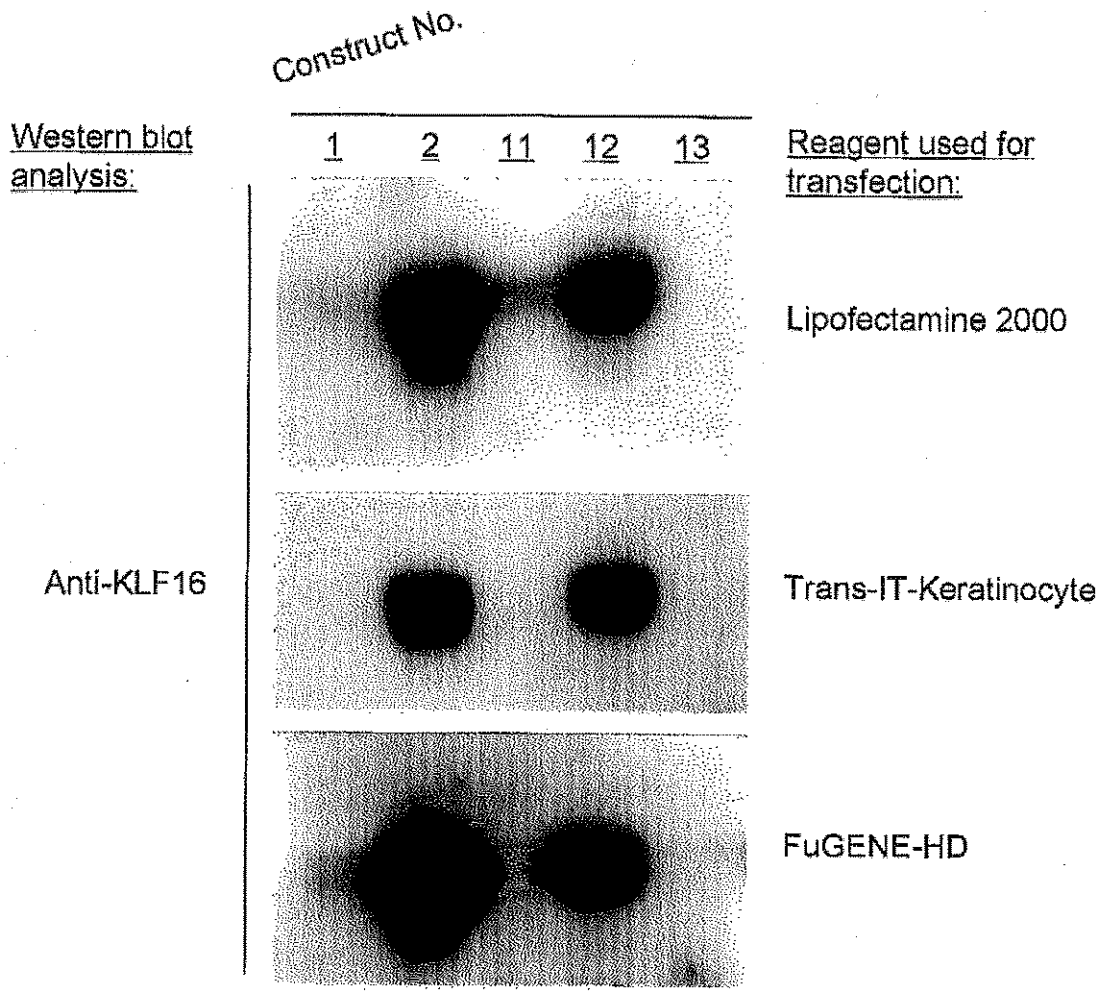
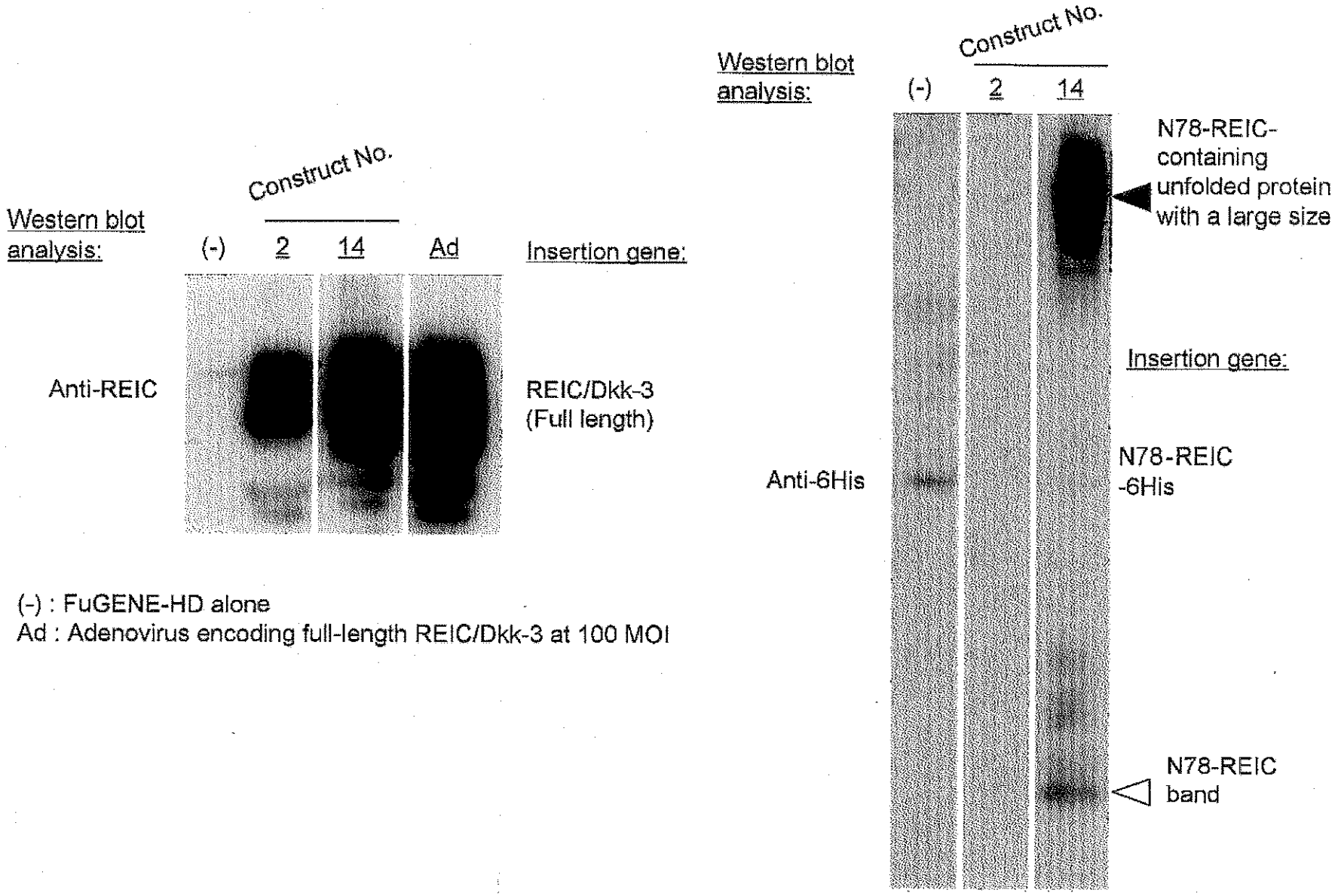

Construct No.
Western blot analysis:
1
2
11
12
13
Reagent used for transfection:
Anti-KLF16
Lipofectamine 2000
Trans-IT-Keratinocyte
FuGENE-HD

Fig. 4



(-) : FuGENE-HD alone
Ad : Adenovirus encoding full-length REIC/Dkk-3 at 100 MOI

Fig. 5-1

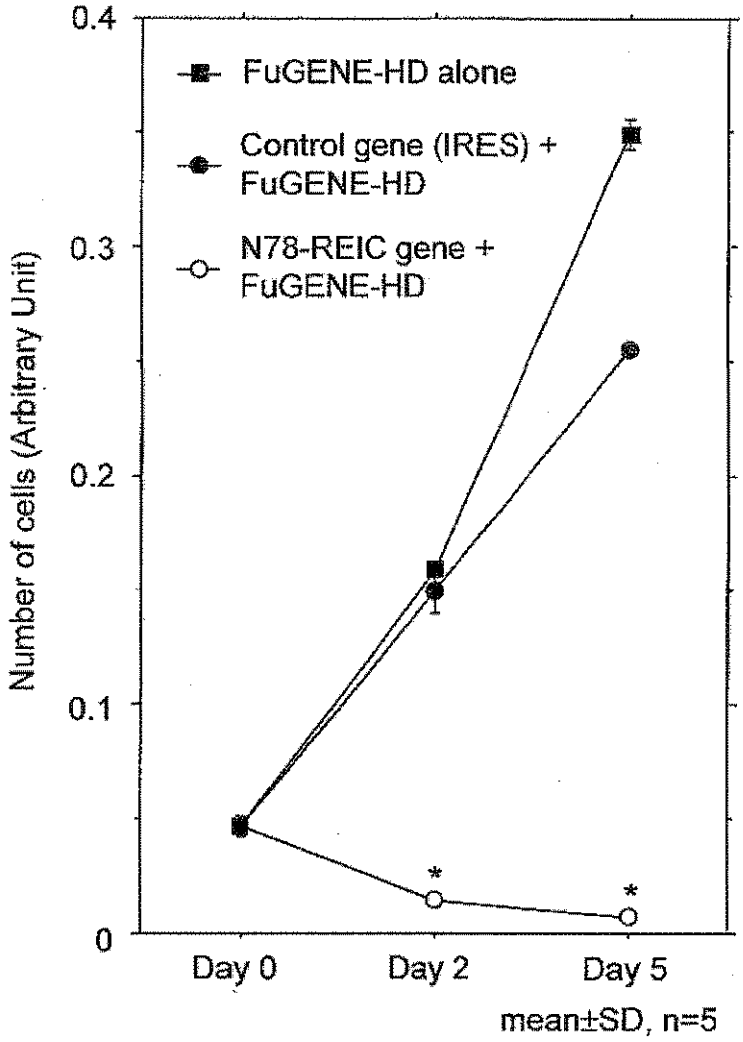
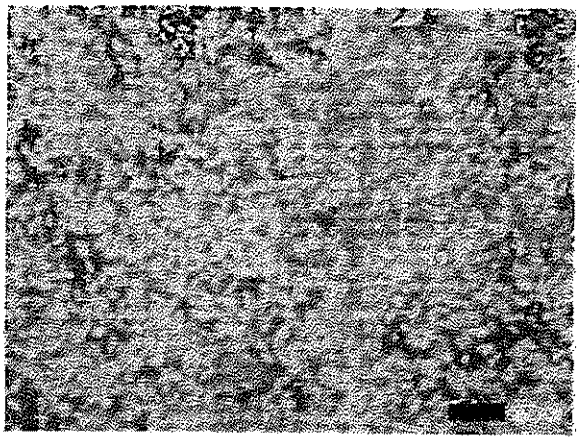

0.4
0.3
0.2
0.1
0
Number of cells (Arbitrary Unit)
FuGENE-HD alone
Control gene (IRES) + FuGENE-HD
N78-REIC gene + FuGENE-HD
*
*
Day 0
Day 2
Day 5
mean±SD, n=5

Fig. 5-2

A

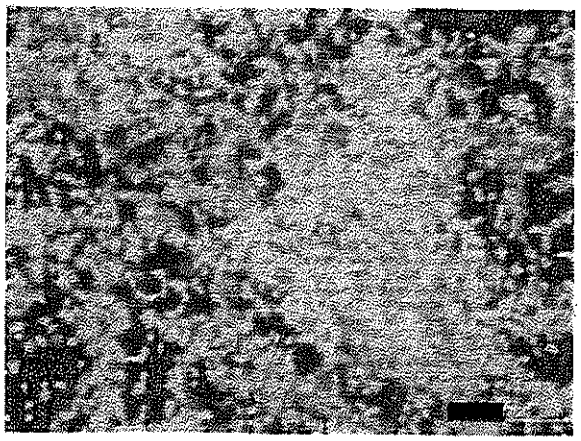
FuGENE-HD alone
(Day 5)



B

Bar = 200 μm

Control gene (IRES)
+ FuGENE-HD
(Day 5)



C

N78-REIC gene
+ FuGENE-HD
(Day 5)

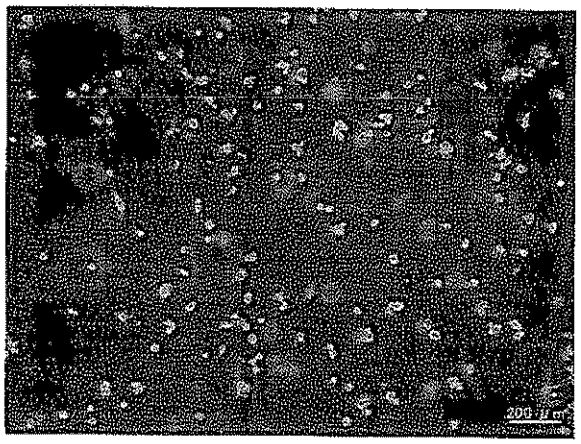
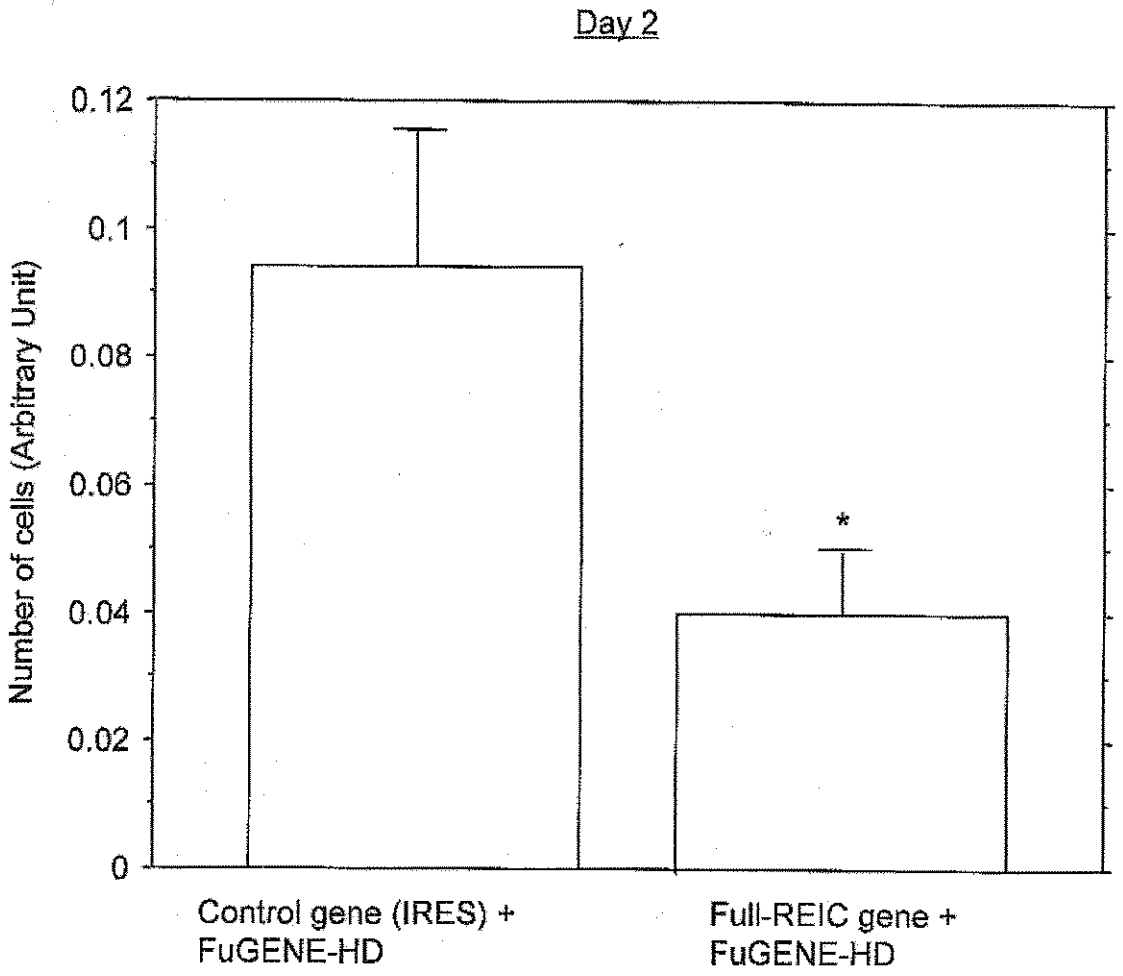


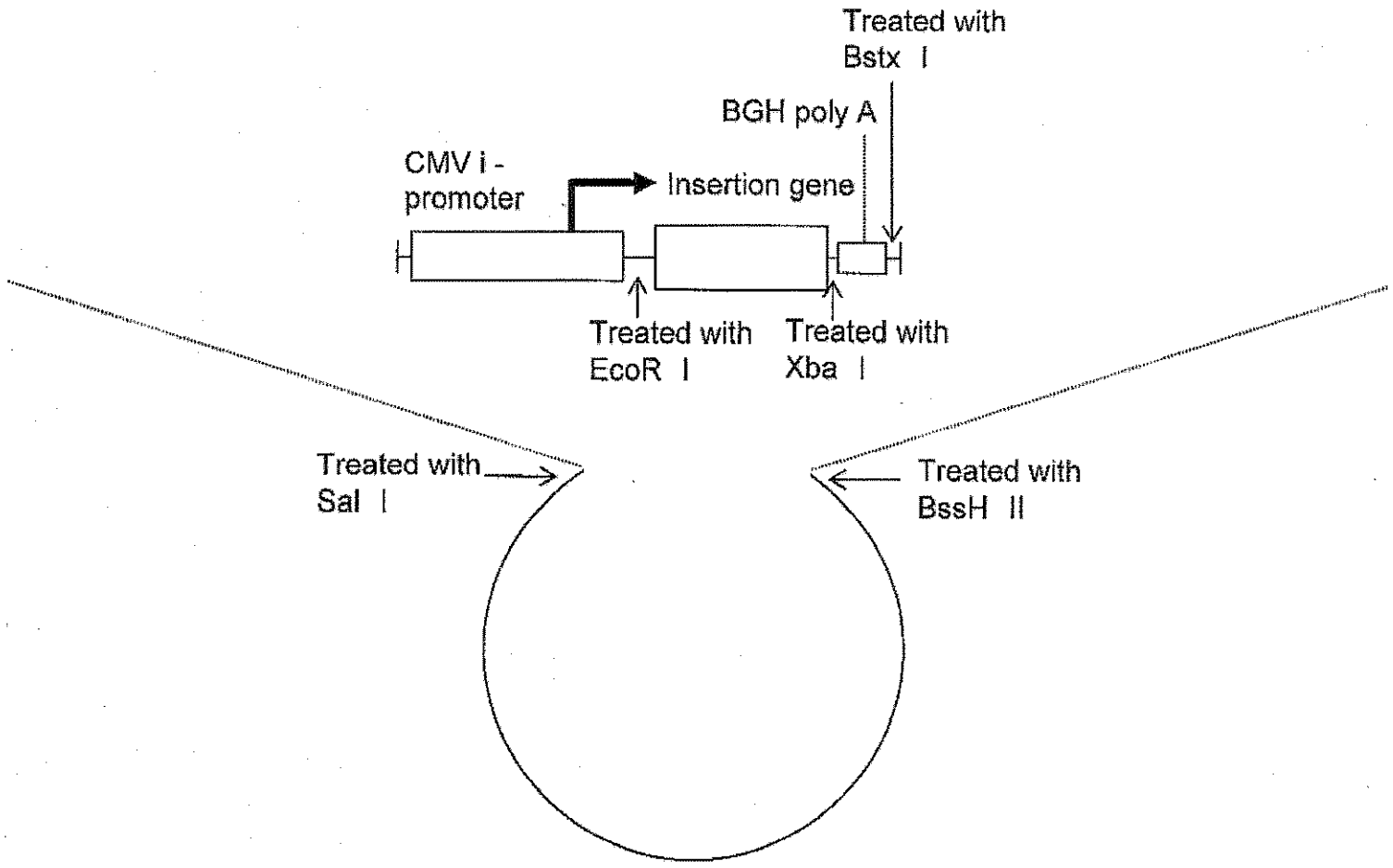
Fig. 6



mean±SE, n=5

Fig. 7

Construct No.1



pDNR-1r Donor vector
(Plasmid cloning vector containing no promoter
(Clontech (Takara Bio)), Product No. PT3616-5)

Fig. 8

Construct No.2

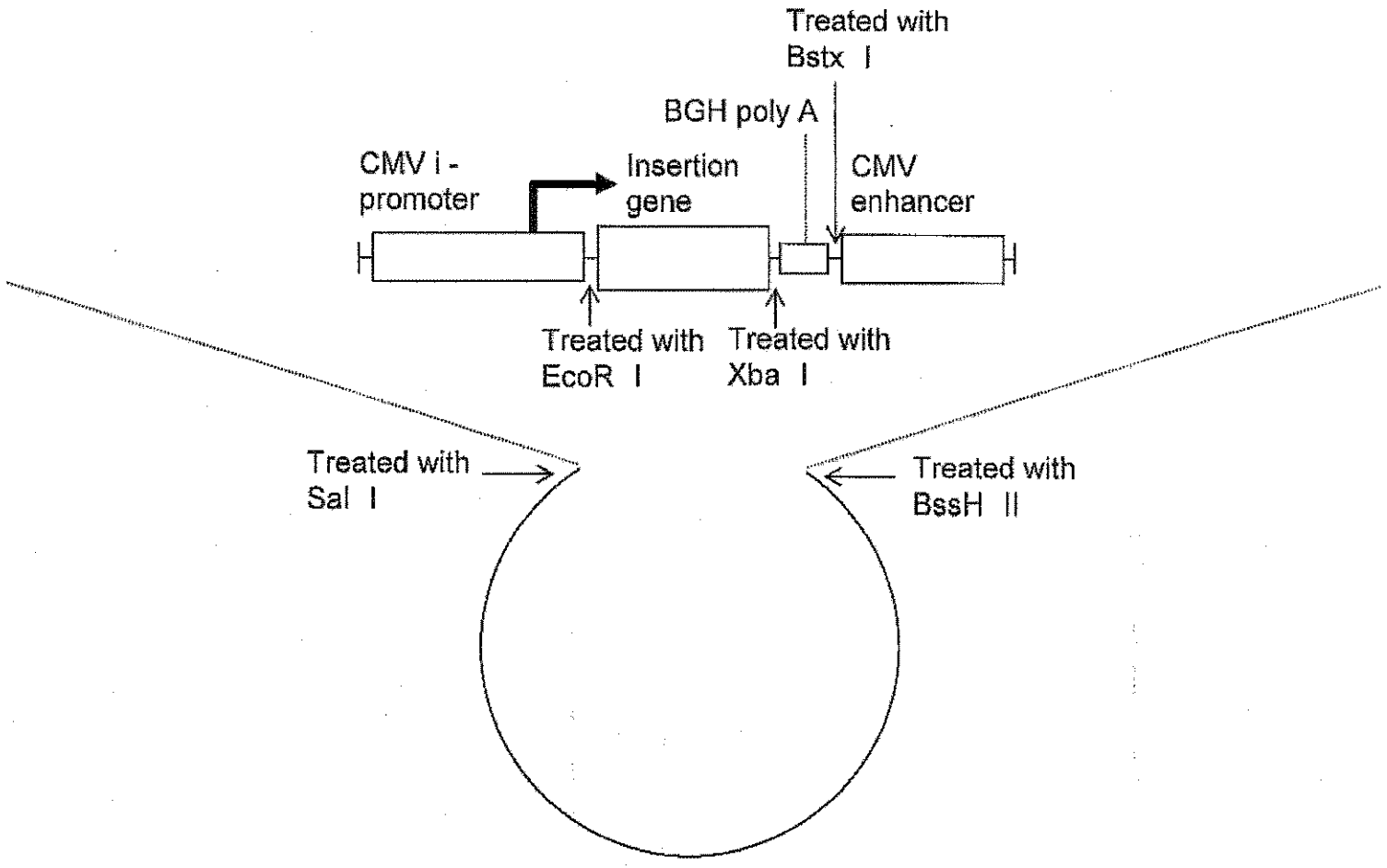


Fig. 9

Construct No.3

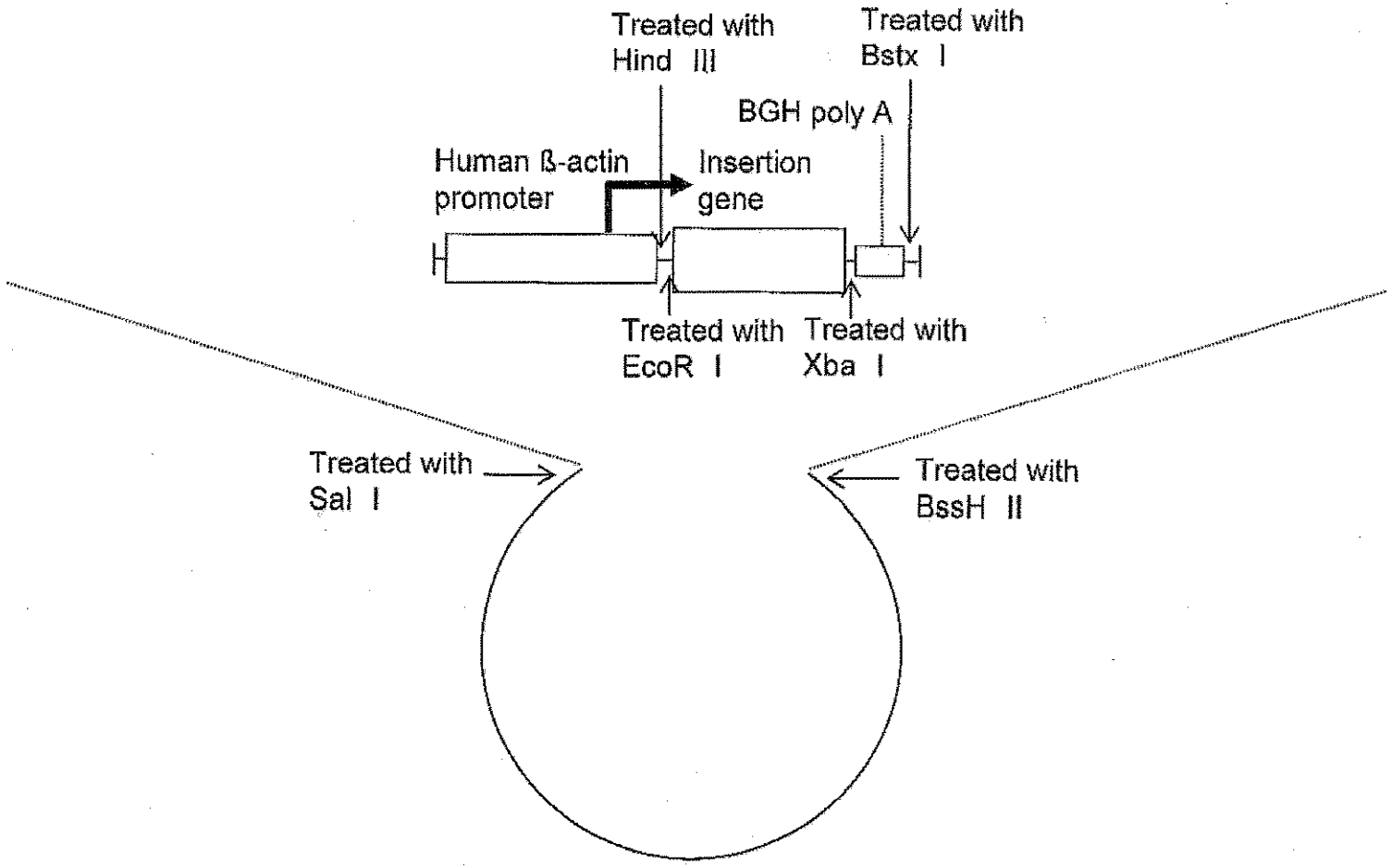
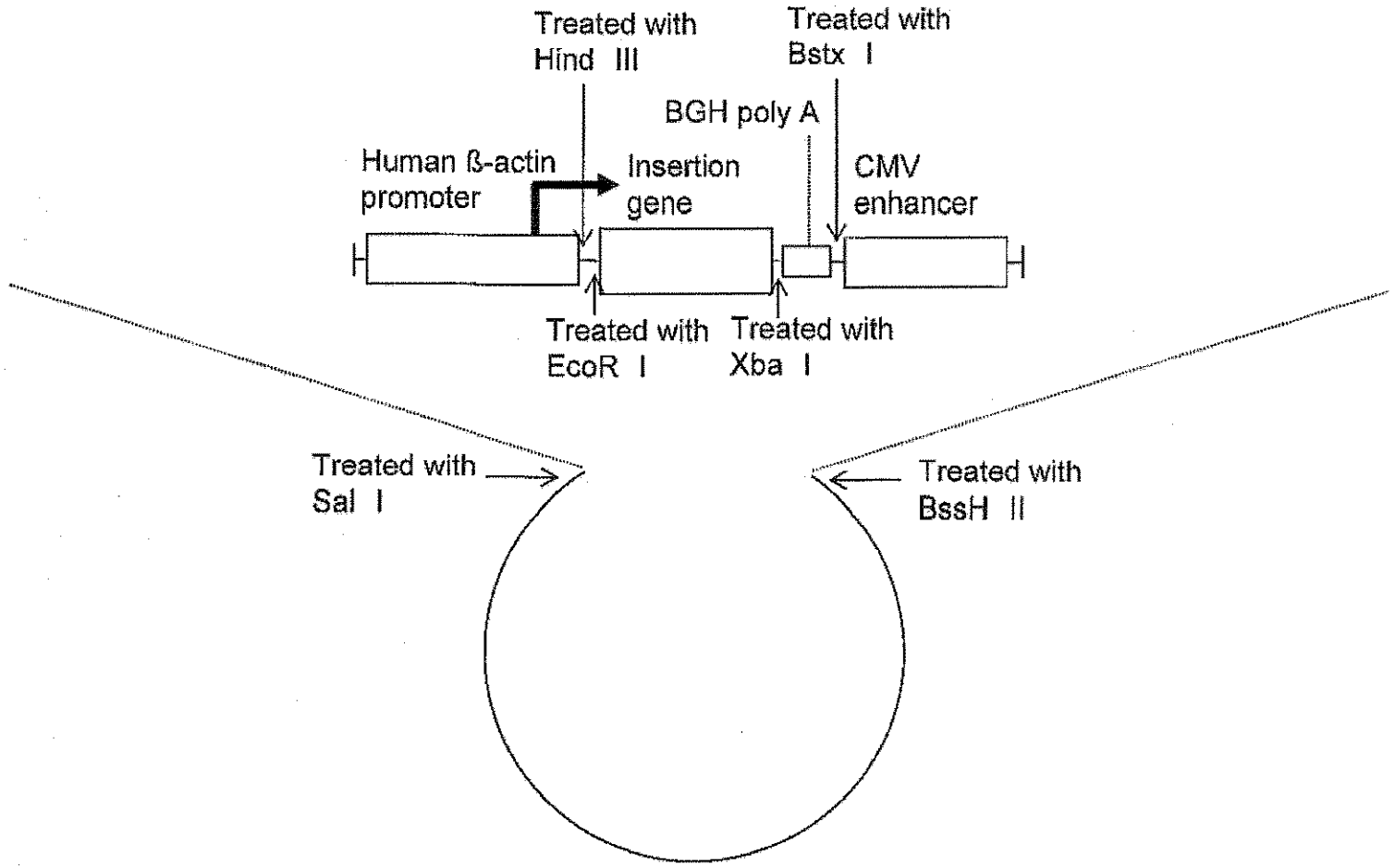


Fig. 10

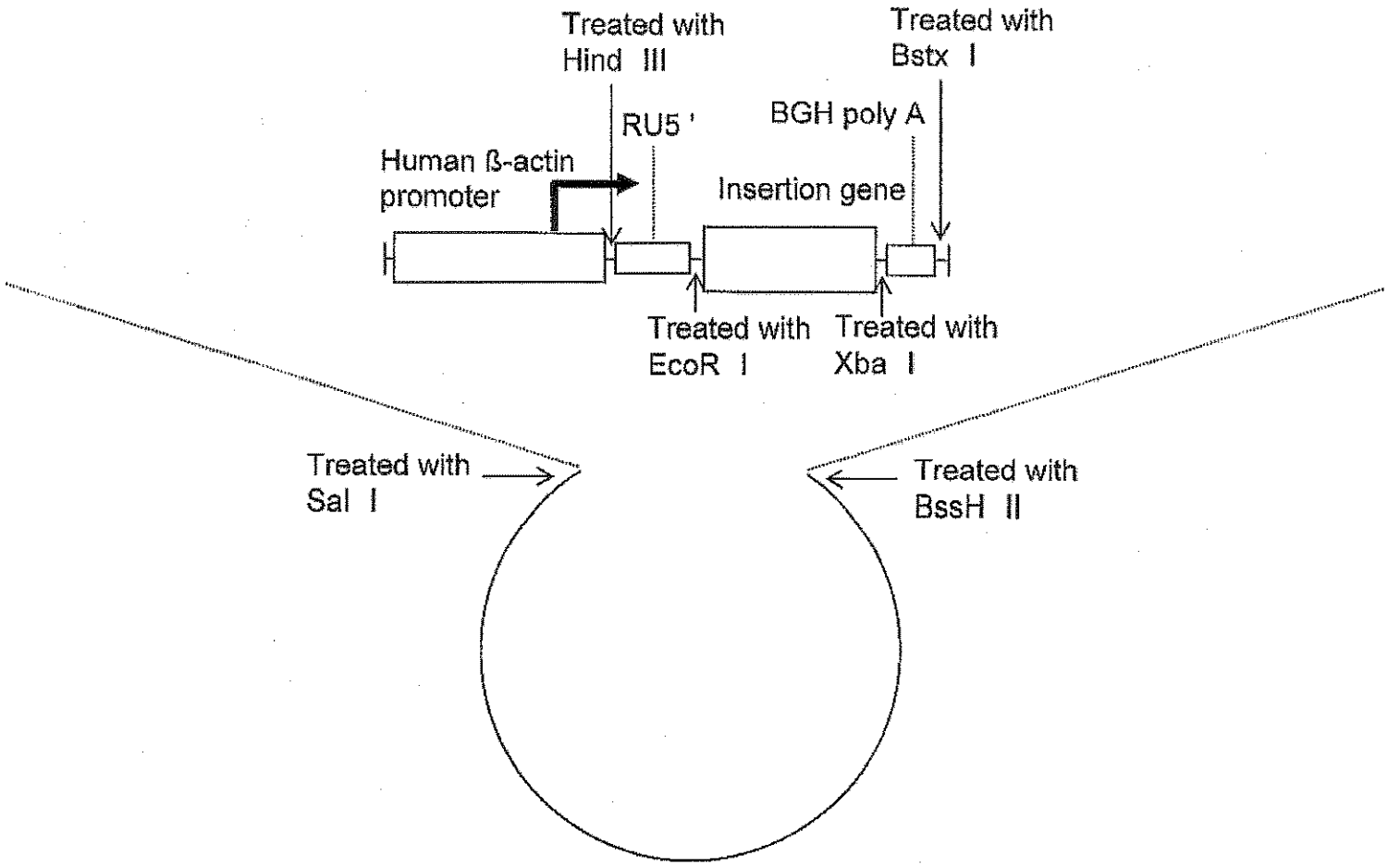
Construct No.4



pDNR-1r Donor vector
(Plasmid cloning vector containing no promoter
(Clontech (Takara Bio)), Product No. PT3616-5)

Fig. 11

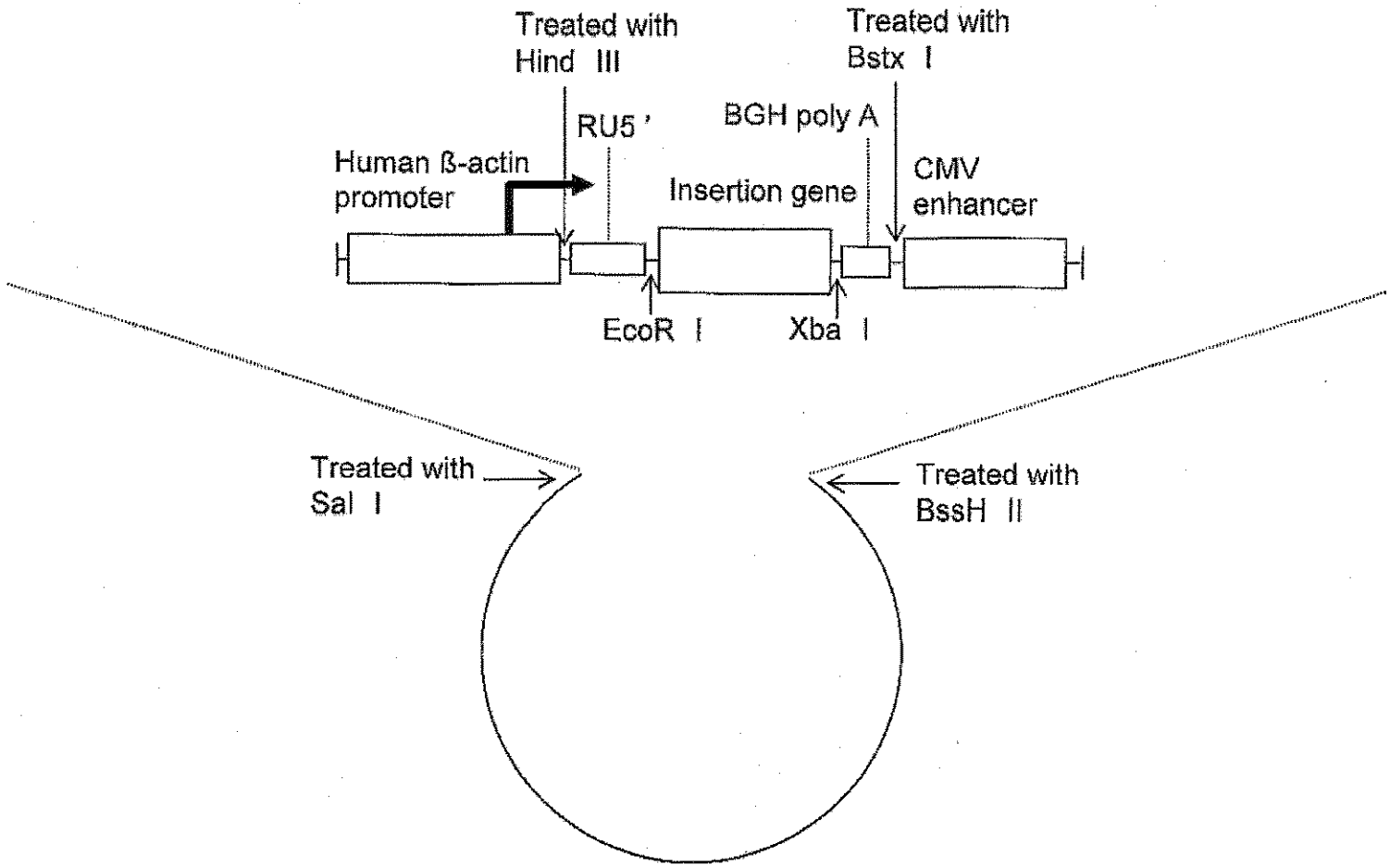
Construct No.5



pDNR-1r Donor vector
(Plasmid cloning vector containing no promoter
(Clontech (Takara Bio)), Product No. PT3616-5)

Fig. 12

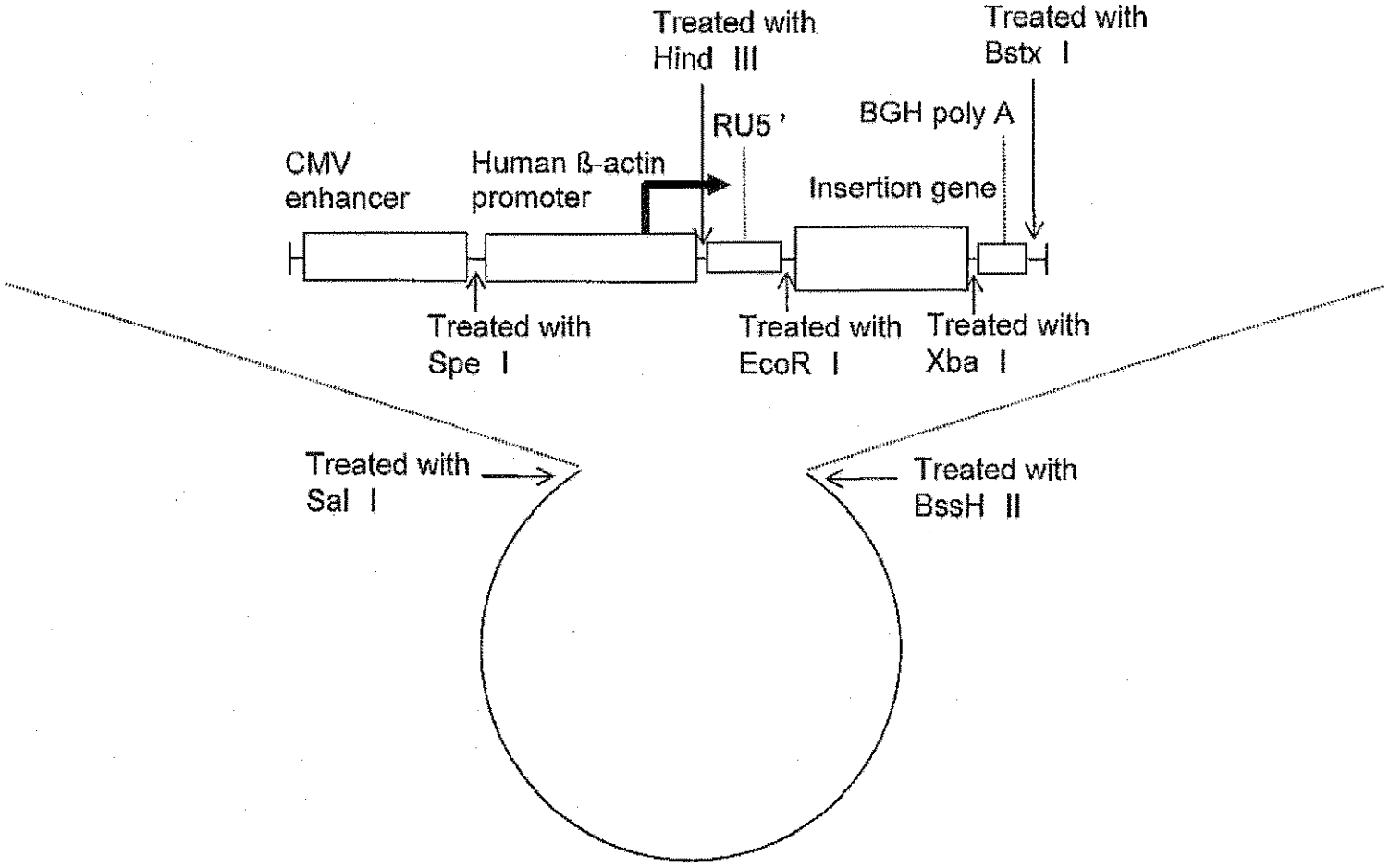
Construct No.6



pDNR-1r Donor vector
(Plasmid cloning vector containing no promoter
(Clontech (Takara Bio)), Product No. PT3616-5)

Fig. 13

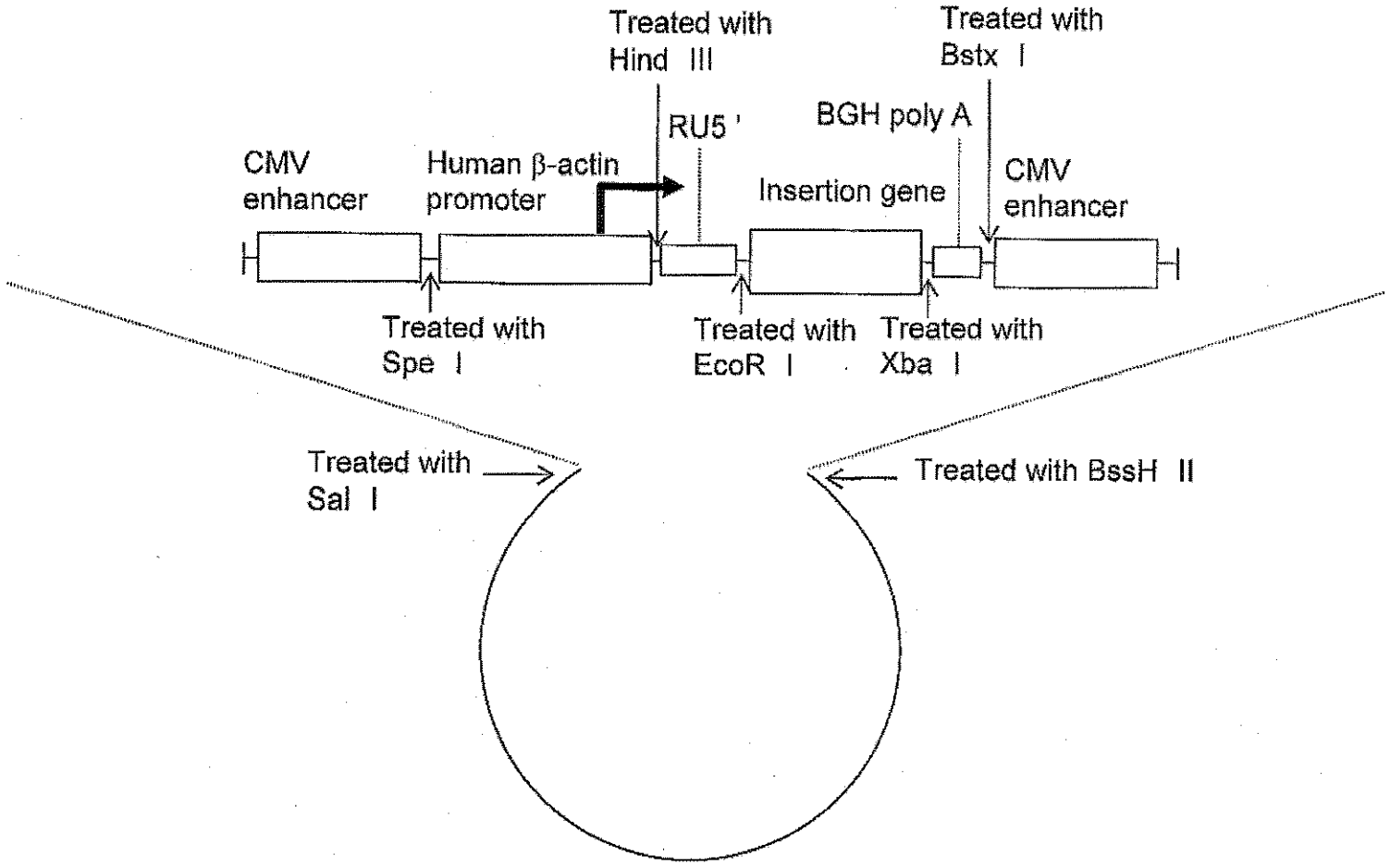
Construct No.7



pDNR-1r Donor vector
(Plasmid cloning vector containing no promoter
(Clontech (Takara Bio)), Product No. PT3616-5)

Fig. 14

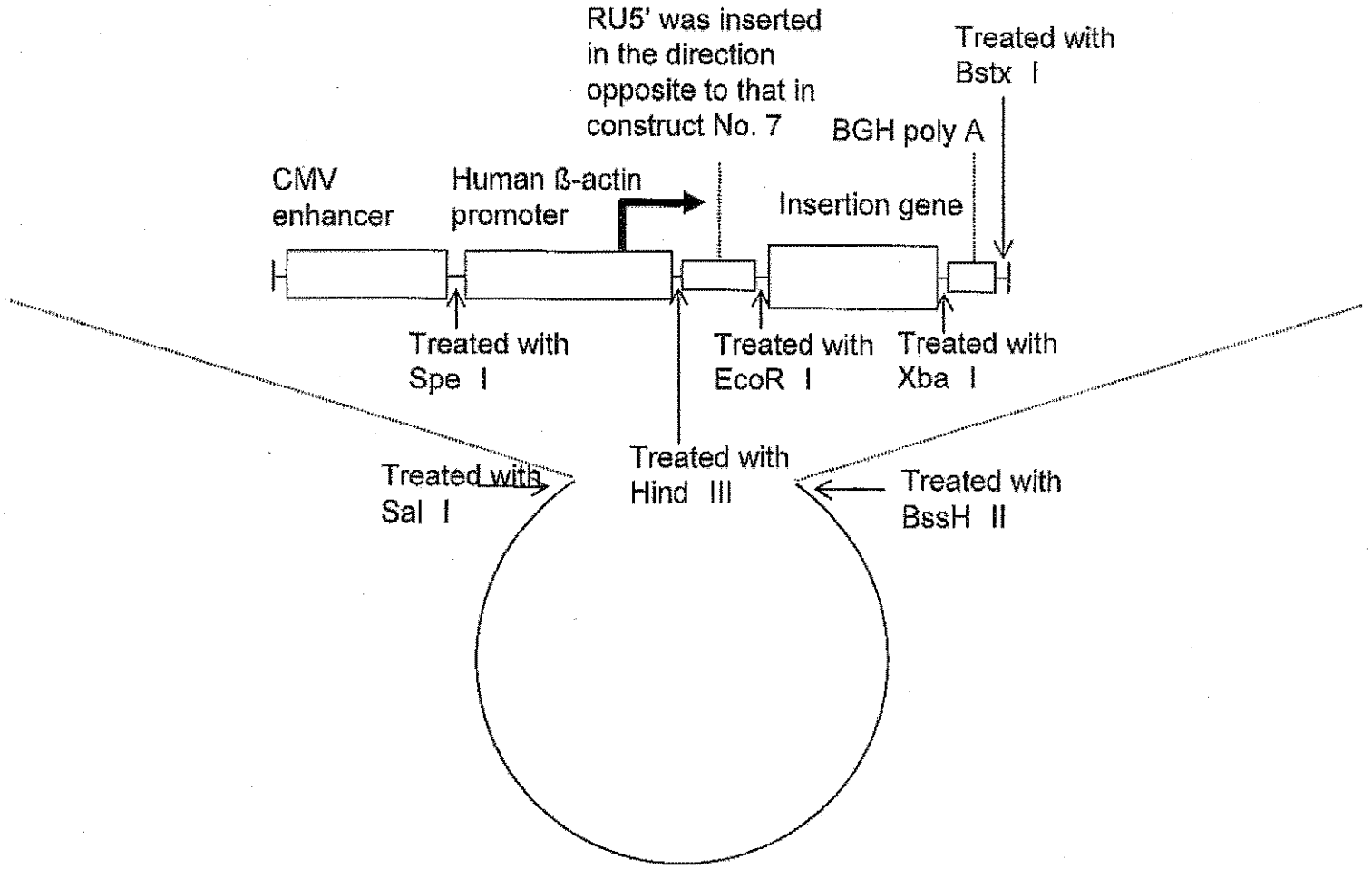
Construct No. 8



pDNR-1r Donor vector
(Plasmid cloning vector containing no promoter
(Clontech (Takara Bio)), Product No. PT3616-5)

Fig. 15

Construct No.9



pDNR-1r Donor vector
(クロンテック社[タカラバイオ社]のpromoterの無い
クローニング用プラスミドベクター, 品番PT3616-5)

Fig. 16

Construct No.10

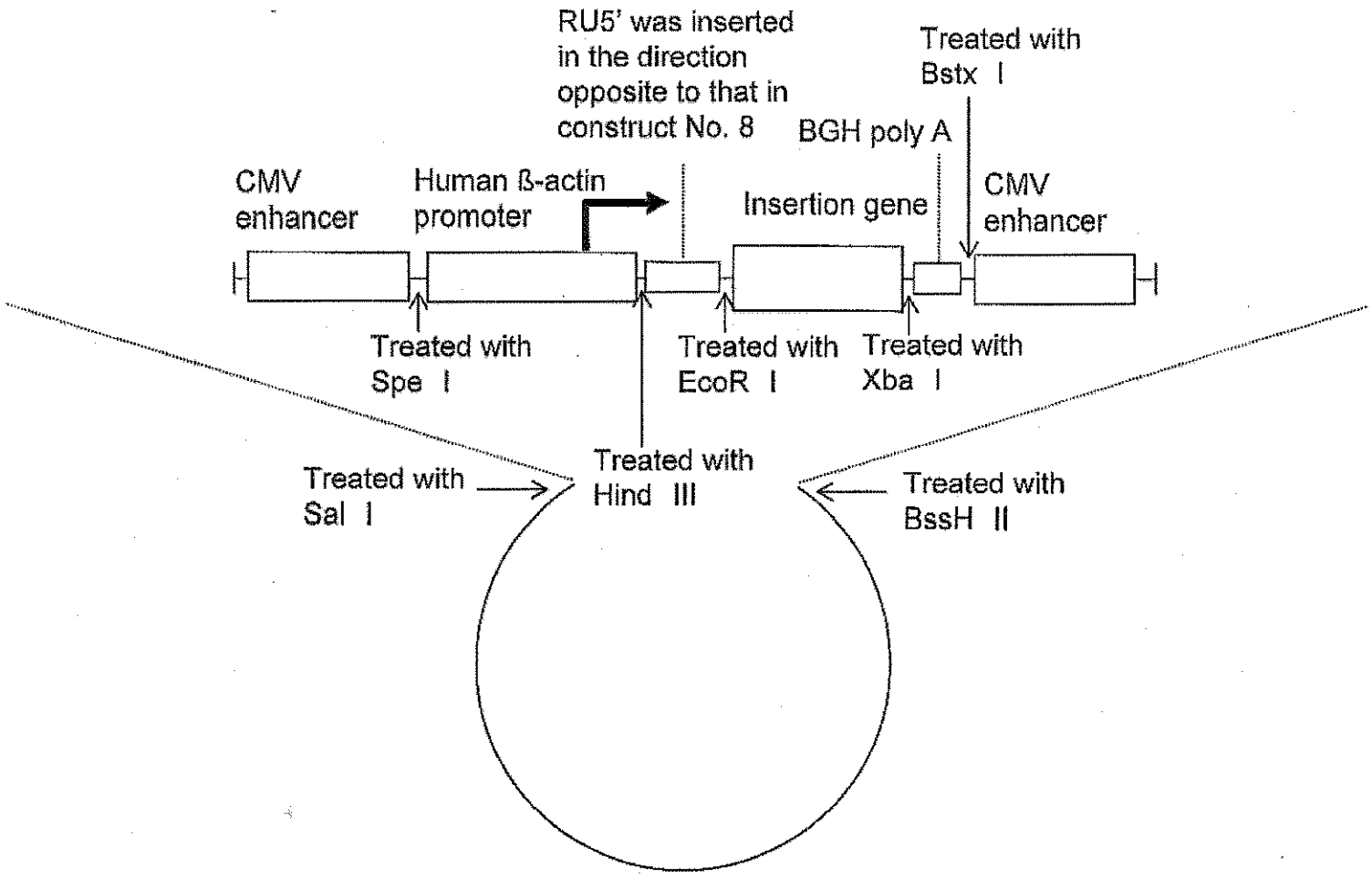
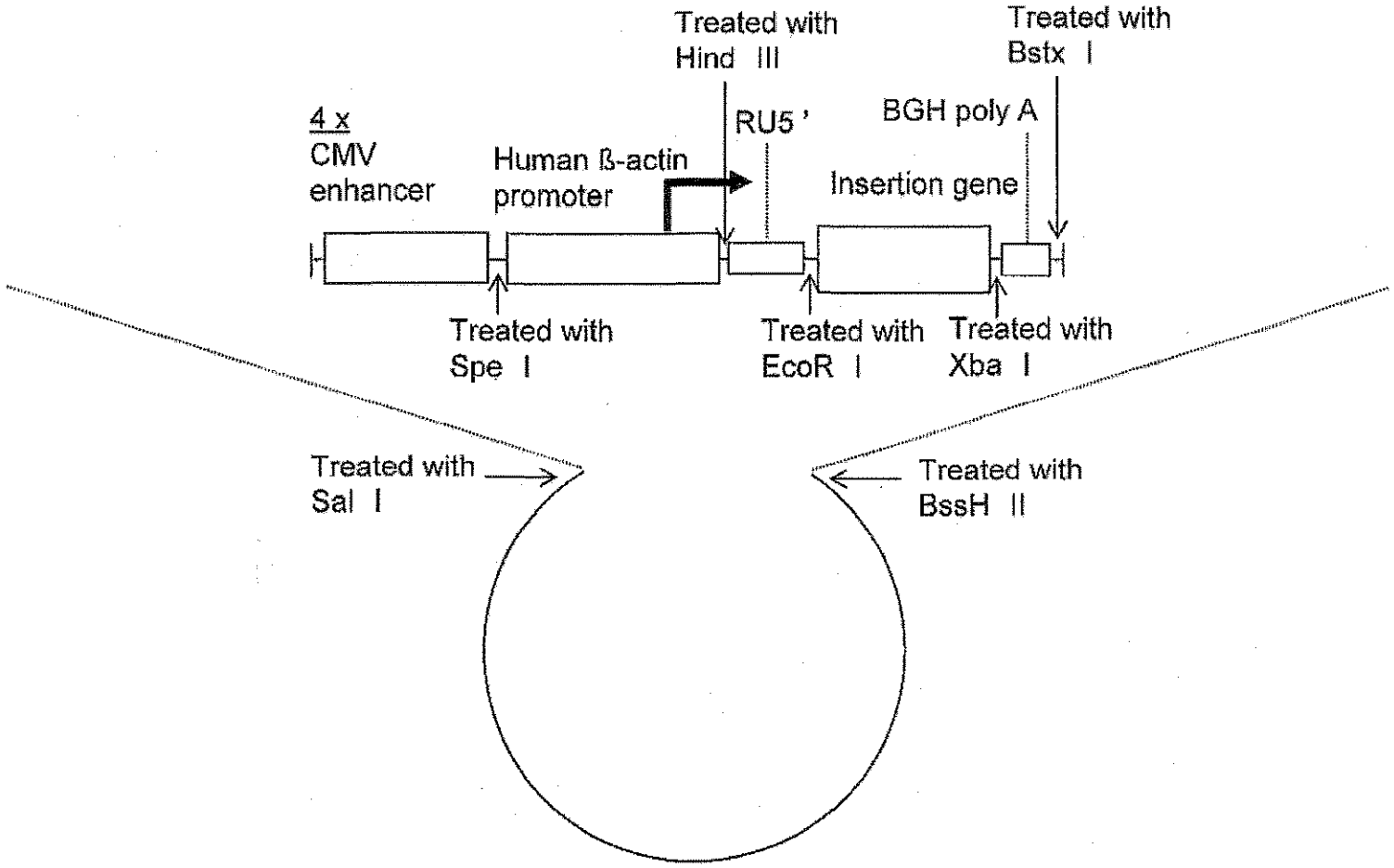


Fig. 17

Construct No.11



pDNR-1r Donor vector
(Plasmid cloning vector containing no promoter
(Clontech (Takara Bio)), Product No. PT3616-5)

Fig. 18

Construct No.12

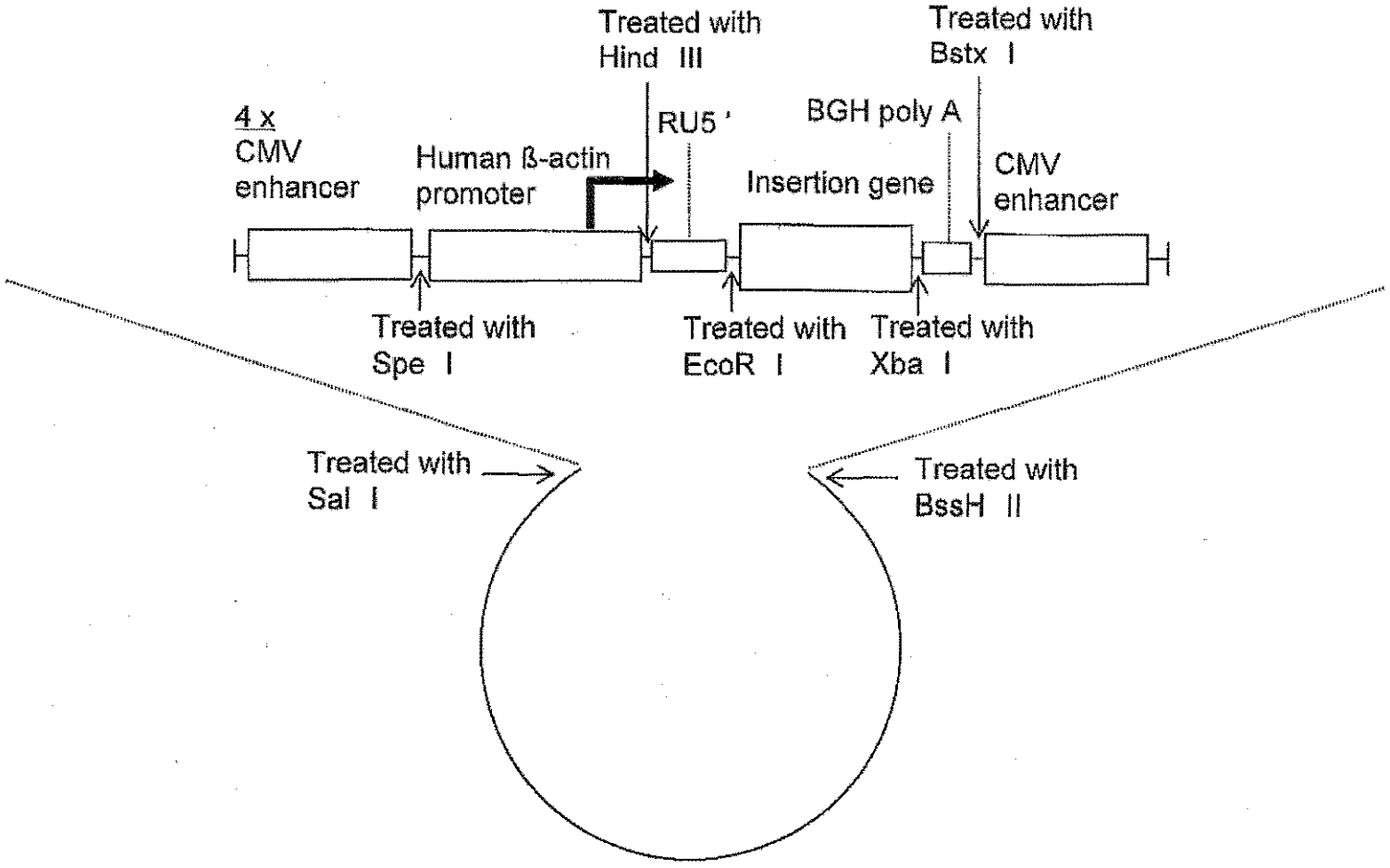


Fig. 19

Construct No.13

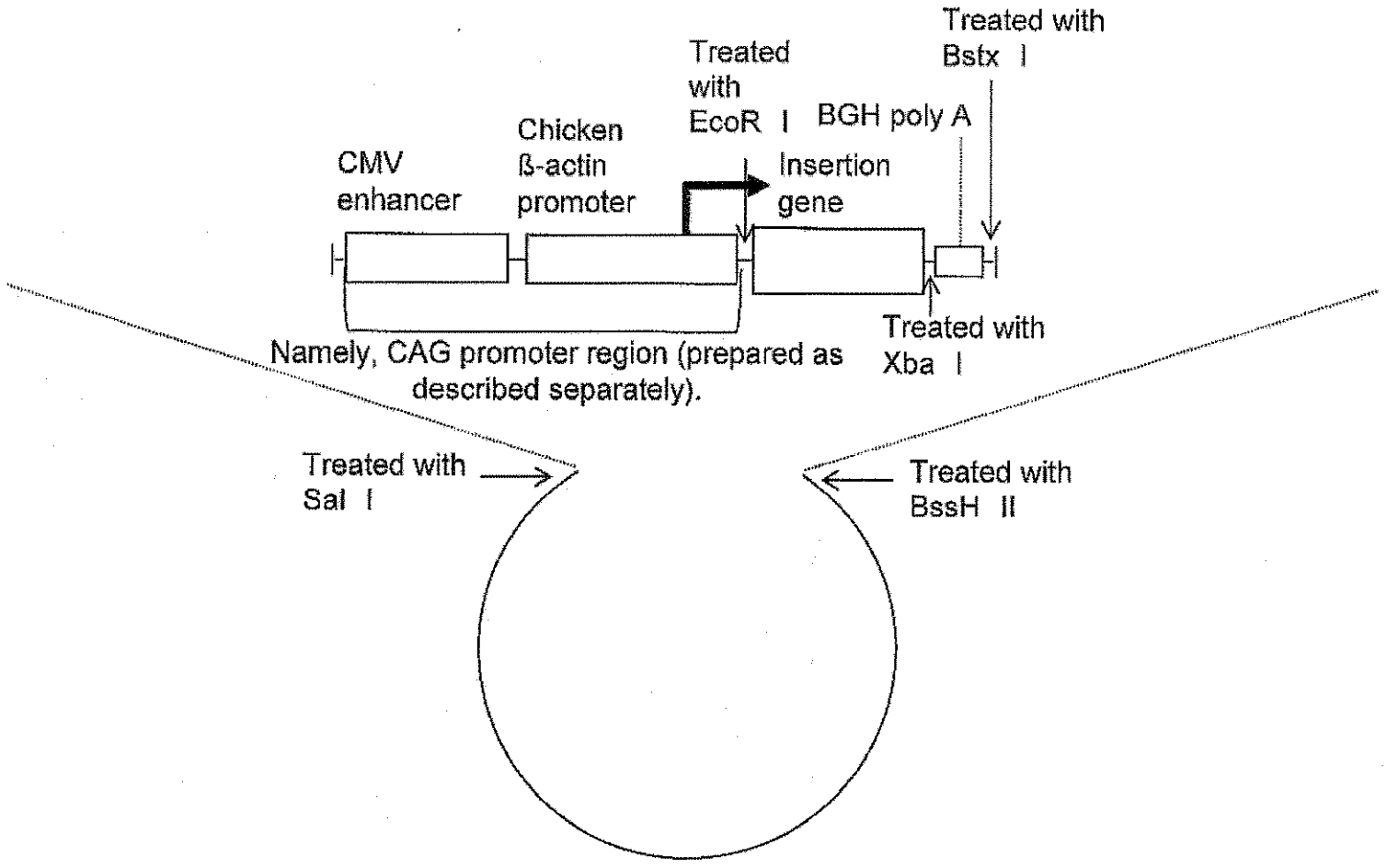


Fig. 20

Construct No.14

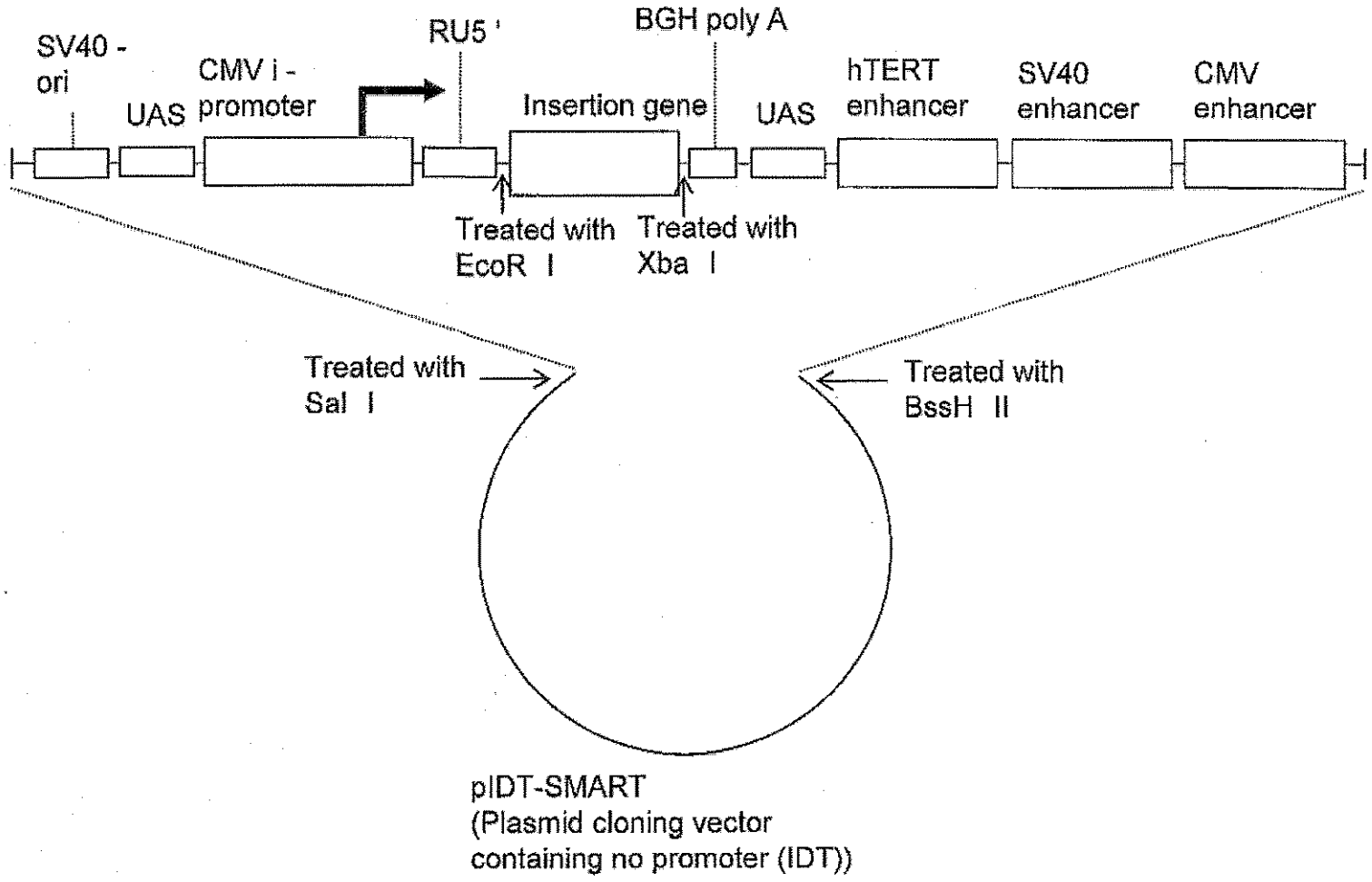


Fig. 21

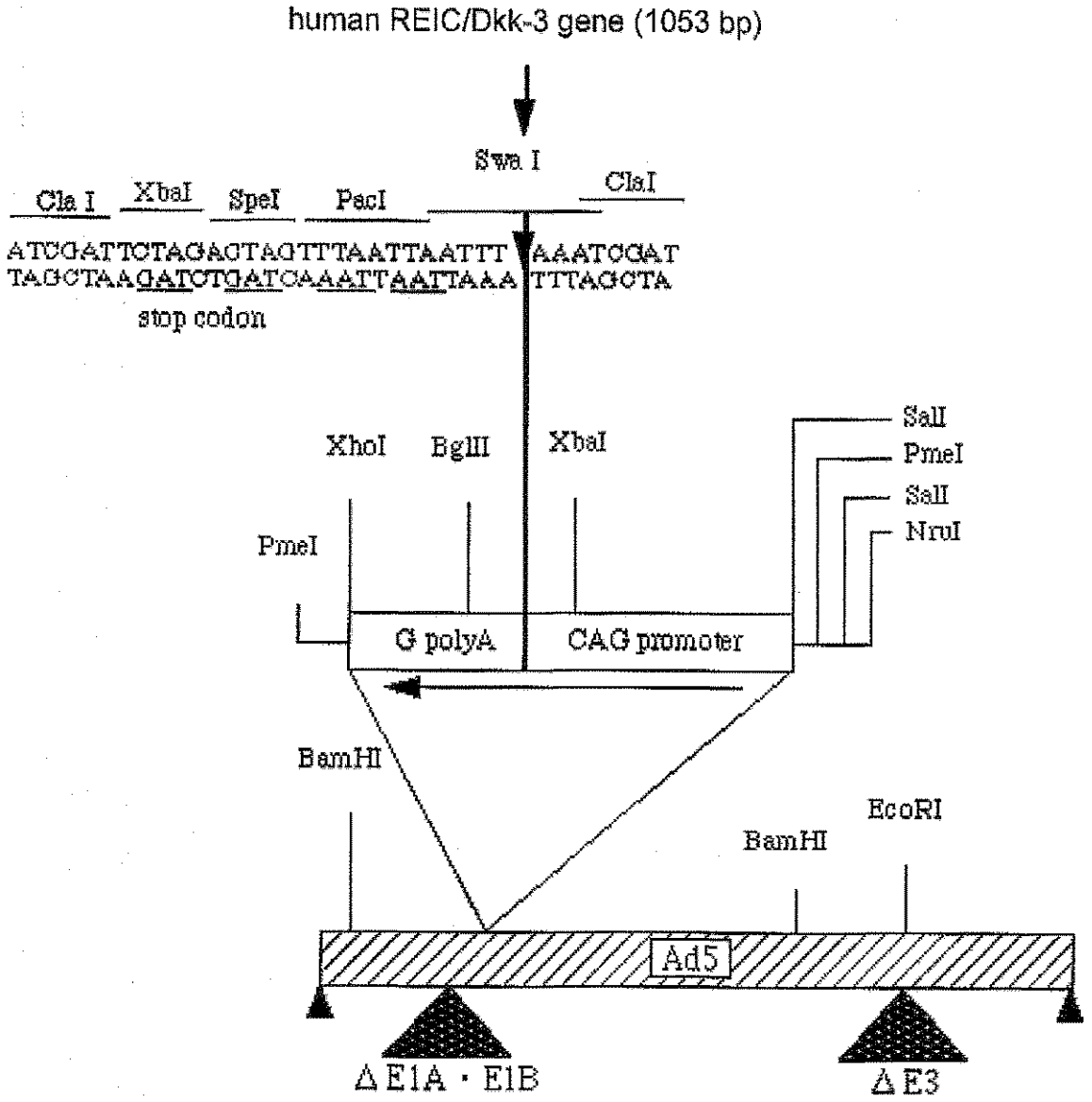

human REIC/Dkk-3 gene (1053 bp)
Swa I
Cla I
XbaI
SpeI
PacI
ClaI
ATCGATTCTAGACTAGTTTAATTAATTT AAATCGAT
TAGCTAAGATCTGATCAAATTAATTAAA TTTAGCTA
stop codon
XhoI
BglII
XbaI
SalI
PmeI
SalI
NruI
PmeI
G polyA
CAG promoter
BamHI
EcoRI
BamHI
Ad5
ΔE1A・E1B
ΔE3

Fig. 22-1

Full nucleotide sequence of pDNR-1r Donor vector

a) Ampicillin resistance
b) Multicloning site in bold character style

GTCGAC: SalI, **GCGCGC**: BssHII

GCGGCCGCATAACTTCGTATAGCATACATTATACGAAGTTAT**CA**GTCGAC Insert a gene to be expressed.

GCGCGC**GGGCCCAGTAGGTAAGTGAA**CATGGTCATAGCTGTTTCCTAGGAGATCCTGGTCAT
GACTAGTGCTTGGATTCTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAA
TCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAACAGGAGTCCAAGCGAGCTCGATAT
CAAATTACGCCCCGCCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTGCCG
ACATGGAAGCCATCACAAACGGCATGATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTC
GCCTTGCGTATAATATTTGCCCATGGTGAAAACGGGGGCGAAGAAGTTGTCCATATTGGCCA
CGTTTAAATCAAAACTGGTGAAACTCACCCAGGGATTGGCTGAGACGAAAAACATATTCTCA
ATAAACCCTTTAGGGAAATAGGCCAGGTTTTCACCGTAACACGCCACATCTTGCGAATATAT
GTGTAGAAACTGCCGGAAATCGTCGTGGTATTCACTCCAGAGCGATGAAAACGTTTCAGTTT
GCTCATGGAAAACGGTGTAACAAGGGTGAACACTATCCCATATCACCAGCTCACCGTCTTTC
ATTGCCATACGAAATTCCGGATGAGCATTCATCAGGCGGGCAAGAATGTGAATAAAGGCCGG
ATAAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCAGCTGAACGG
TCTGGTTATAGGTACATTGTGTGATTAAAAAGGCAACTTTATGCCCATGCAACAGAAACTAT
AAAAAATACAGAGAATGAAAAGAAACAGATAGATTTTTTAGTTCTTTAGGCCCGTAGTCTGC
AAATCCTTTTATGATTTTCTATCAAACAAAAGAGGAAAATAGACCAGTTGCAATCCAAACGA
GAGTCTAATAGAATGAGGTCGAAAAGTAAATCGCGCGGGTTTGTTACTGATAAAGCAGGCAA
GACCTAAAATGTGTAAAGGGCAAAGTGTATACTTTGGCGTCACCCCTTACATATTTTAGGTC
TTTTTTTATTGTGCGTAACTAACTTGCCATCTTCAAACAGGAGGGCTGGAAGAAGCAGACCG
CTAACACAGTACATAAAAAAGGAGACATGAACGATGAACATCAAAAAGTTTGCAAAACAAGC
AACAGTATTAACCTTTACTACCGCACTGCTGGCAGGAGGCGCAACTCAAGCGTTTGCGAAAG
AAACGAACCAAAAGCCATATAAGGAAACATACGGCATTTCCCATATTACACGCCATGATATG
CTGCAAATCCCTGAACAGCAAAAAAATGAAAAATATCAAGTTCCTGAGTTCGATTCGTCCAC
AATTAAAAATATCTCTTCTGCAAAAGGCCTGGACGTTTGGGACAGCTGGCCATTACAAAACG
CTGACGGCACTGTCGCAAACTATCACGGCTACCACATCGTCTTTGCATTAGCCGGAGATCCT
AAAAATGCGGATGACACATCGATTTACATGTTCTATCAAAAAGTCGGCGAAACTTCTATTGA
CAGCTGGAAAAACGCTGGCCGCGTCTTTAAAGACAGCGACAAATTCGATGCAAATGATTCTA
TCCTAAAAGACCAAACACAAGAATGGTCAGGTTCAGCCACATTTACATCTGACGGAAAAATC
CGTTTATTCTACACTGATTTCTCCGGTAAACATTACGGCAAACAAACACTGACAACTGCACA
AGTTAACGTATCAGCATCAGACAGCTCTTTGAACATCAACGGTGTAGAGGATTATAAATCAA
TCTTTGACGGTGACGGAAAAACGTATCAAAATGTACAGCAGTTCATCGATGAAGGCAACTAC
AGCTCAGGCGACAACCATACGCTGAGAGATCCTCACTACGTAGAAGATAAAGGCCACAAATA
CTTAGTATTTGAAGCAAACACTGGAACTGAAGATGGCTACCAAGGCGAAGAATCTTTATTTA

Fig. 22-2

ACAAAGCATACTATGGCAAAAGCACATCATTCTTCCGTCAAGAAAGTCAAAAACTTCTGCAA
AGCGATAAAAAACGCACGGCTGAGTTAGCAAACGGCGCTCTCGGTATGATTGAGCTAAACGA
TGATTACACACTGAAAAAAGTGATGAAACCGCTGATTGCATCTAACACAGTAACAGATGAAA
TTGAACGCGCGAACGTCTTTAAAATGAACGGCAAATGGTACCTGTTCACTGACTCCCGCGGA
TCAAAAATGACGATTGACGGCATTACGTCTAACGATATTTACATGCTTGGTTATGTTTCTAA
TTCTTTAACTGGCCCATACAAGCCGCTGAACAAAACTGGCCTTGTGTTAAAAATGGATCTTG
ATCCTAACGATGTAACCTTTACTTACTCACACTTCGCTGTACCTCAAGCGAAAGGAAACAAT
GTCGTGATTACAAGCTATATGACAAACAGAGGATTCTACGCAGACAAACAATCAACGTTTGC
GCCTAGCTTCCTGCTGAACATCAAAGGCAAGAAAACATCTGTTGTCAAAGACAGCATCCTTG
AACAAGGACAATTAACAGTTAACAAATAAAAACGCAAAAGAAAATGCCGATATCCTATTGGC
ATTGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCT
AAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATAT
TGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGC
ATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATC
AGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGT
TTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGT
ATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATG
ACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAA
TTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGAT
CGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTG
ATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCT
GTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCG
GCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCC
TTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATC
ATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAG
TCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGC
ATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTT
TAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACG
TGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATC
CTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTT
TGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCA
GATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAG
CACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAG
TCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTG
AACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACC
TACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCG
GTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTA
TCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGT
CAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTT
TGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTAT
TACCGCCTTACGCGTGTAAAACGACGGCCAGTAGATCTGTAATACGACTCACTATAGGGCGC
TAGCTGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAA

Fig. 23

Full nucleotide sequence of pIDT-SMART vector

a) Kanamycin resistance
b) Multicloning site in bold character style

GATATC: EcoRV

GGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTC
TTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATT
AGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTA
CACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTCGGAAAAAGAGT
TGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGC
AGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATTTTCTACCGAAGAAAG
GCCCACCCGTGAAGGTGAGCCAGTGAGTTGATTGCAGTCCAGTTACGCTGGAGTCTGAGGCT
CGTCCTGAATGTGTAAAACGACGGCCAGTTTATCTAGTCAGCTTGATTCTAGCTGATCGTGG
ACCGGAAGGTGAGCCAGTGAGTTGATTGCAGTCCAGTTACGCTGGAGTCTGAGGCTCGTCCT
GAATGATAT**ACGCGTCGGAGGGTTGCGTTTGAGACGGGCGACA**GAT Insert a gene to be expressed.
ATCAGTTCTGGACGAGCGAGCTGTCGTCCGGCGGCCGCGATCTTACGGCATTATACGTATGA
TCGGTCCACGATCAGCTAGATTATCTAGTCAGCTTGATGTCATAGCTGTTTCCTGAGGCTCA
ATACTGACCATTTAAATCATACCTGACCTCCATAGCAGAAAGTCAAAAGCCTCCGACCGGAG
GCTTTTGACTTGATCGGCACGTAAGAGGTTCCAACTTTCACCATAATGAAATAAGATCACTA
CCGGGCGTATTTTTTGAGTTATCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGAGCCATAT
TCAACGGGAAACGTCTTGCTTGAAGCCGCGATTAAATTCCAACATGGATGCTGATTTATATG
GGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGG
AAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTAC
AGATGAGATGGTCAGGCTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATT
TTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCAGGGAAAACAGCATTC
CAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCT
GCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACGGCGATCGCGTATTTCGTC
TCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGGTGCGAGTGATTTTGATGACGAG
CGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTCTTGCCATTCTCACC
GGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAAT
TAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATC
CTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGG
TATTGATAATCCTGATATGAATAAATTGCAGTTTCACTTGATGCTCGATGAGTTTTTCTAAT
GAGGACCTAAATGTAATCACCTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTGCTGGCGTTT
TTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGATGCTCAAGTCAGAGGTGGCG
AAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTC
CTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCG
CTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCT

Fig. 24

Nucleotide sequence of CMVi promoter (hCMV + intron promoter) region

tccgcgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgccca
ttgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtca
atgggtggagtatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaa
gtacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatg
accttatgggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggt
gatgcggttttggcagtacatcaatgggcgtggatagcggtttgactcacggggatttccaa
gtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggactttcca
aaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtgggaggt
ctatataagcagagctcgtttagtgaaccgtcagatcgcctggagacgccatccacgctgtt
ttgacctccatagaagacaccgggaccgatccagcctccgcggccgggaacggtgcattgga
acgcggattccccgtgccaagagtgacgtaagtaccgcctatagactctataggcacacccc
tttggctcttatgcatgaattaatacgactcactatagggagacagactgttcctttcctgg
gtcttttctg

Fig. 25

Nucleotide sequence of BGH polyA (3 x stop + BGH poly A) region

*tgactgactgac*GTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATC
TGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTT
CC**TAATAAA**ATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGT
GGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGC
GGTGGGCTCTATGG

Fig. 26

Nucleotide sequence of CMV enhancer region

Tccgcgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgccca
ttgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtca
atgggtggagtatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaa
gtacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatg
accttatgggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggt

Fig. 27

Nucleotide sequence of human β-actin promoter region

gttccatgtccttatatggactcatctttgcctattgcgacacacactcagtgaacacctac
tacgcgctgcaaagagccccgcaggcctgaggtgcccccacctcaccactcttcctatttttt
gtgtaaaaatccagcttcttgtcaccacctccaaggaggggggaggaggaggaaggcaggttc
ctctaggctgagccgaatgcccctctgtggtcccacgccactgatcgctgcatgcccaccac
ctgggtacacacagtctgtgattcccggagcagaacggaccctgcccacccggtcttgtgtg
ctactcagtggacagacccaaggcaagaaagggtgacaaggacagggtcttcccaggctggc
tttgagttcctagcaccgccccgcccccaatcctctgtggcacatggagtcttggtccccag
agtcccccagcggcctccagatggtctgggagggcagttcagctgtggctgcgcatagcaga
catacaacggacggtgggcccagacccaggctgtgtagacccagcccccccgccccgcagtg
cctaggtcacccactaacgccccaggccttgtcttggctgggcgtgactgttaccctcaaaa
gcaggcagctccagggtaaaaggtgccctgccctgtagagcccaccttccttcccagggctg
cggctgggtaggtttgtagccttcatcacgggccacctccagccactggaccgctggccccct
gccctgtcctggggagtgtggtcctgcgacttctaagtggccgcaagccacctgactccccc
aacaccacactctacctctcaagcccaggtctctccctagtgacccacccagcacatttagc
tagctgagccccacagccagaggtcctcaggccctgctttcagggcagttgctctgaagtcg
gcaaggggagtgactgcctggccactccatgccctccaagagctccttctgcaggagcgta
cagaacccagggccctggcacccgtgcagaccctggcccacccccacctgggcgctcagtgcc
caagagatgtccacacctaggatgtcccgcggtgggtgggggcccgagagacgggcaggcc
gggggcaggcctggccatgcggggccgaaccgggcactgcccagcgtggggcgcgggggcca
cggcgcgcgcccccagcccccgggcccagcaccccaaggcggccaacgccaaaactctccct
cctcctcttcctcaatctcgctctcgctctttttttttttcgcaaaaggaggggagagggggg
taaaaaaatgctgcactgtgcggcgaagccggtgagtgagcggcgcggggccaatcagcgtg
cgccgttccgaaagttgccttttatggctcgagcggccgcggcggcgccctataaaacccag
cggcgcgacgcgccaccaccgccgagaccgcgtccgccccgcgagcacagagcctcgccttt
gccgatccgccgcccgtcca

Fig. 28

Nucleotide sequence of RU5' forward {R segment of HTLV Type 1 long terminal repeat and a portion (R-U5') of the U5 sequence } region

agcttcgaggggctcgcatctctccttcacgcgcccgccgccctacctgaggccgccatcca
cgccggttgagtcgcgttctgccgcctcccgcctgtggtgcctcctgaactgcgtccgccgt
ctaggtaagtttaaagctcaggtcgagaccgggcctttgtccggcgctcccttggagcctac
ctagactcagccggctctccacgctttgcctgaccctgcttgctcaactctacgtctttgtt
tcgttttctgttctgcgccgttacagatccaagccacc

Fig. 29

Nucleotide sequence of RU5' reverse {R segment of HTLV Type 1 long terminal repeat and a portion (R-U5') of the U5 sequence } region

ggtggcttggatctgtaacggcgcagaacagaaaacgaaacaaagacgtagagttgagcaag
cagggtcaggcaaagcgtggagagccggctgagtctaggtaggctccaagggagcgccggac
aaaggcccggtctcgacctgagctttaaacttacctagacggcggacgcagttcaggaggca
ccacaggcgggaggcggcagaacgcgactcaaccggcgtggatggcggcctcaggtagggcg
gcgggcgcgtgaaggagagatgcgagcccctcgaagct

Fig. 30

Nucleotide sequence of 4 x CMV enhancer region

gtcgacgtcgccccattgacgtcaatgggcgttacataacttacggtaaatggcccgcctgg
ctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgc
caatagggactttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggca
gtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacggtaaatggcc
cgcctggcattatgcccagtacatgaccttatgggactttcctacttggcagtacatctacg
tattagtcatcgctattaccatggtccccattgacgtcaatgggcgttacataacttacggt
aaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatg
ttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaa
actgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaa
tgacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctactt
ggcagtacatctacgtattagtcatcgctattaccatggtccccattgacgtcaatgggcgt
tacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgt
caataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtg
gagtatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgcc
ccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttat
gggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggtccccatt
gacgtcaatgggGGACTAGT

Fig. 31

Nucleotide sequence of CAG promoter region

ATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCC
CCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATT
GACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCAT
ATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCA
GTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTA
CCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCC
CCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGG
CGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGC
GGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGC
GGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGCGCTGCCTTCGCCCCGTG
CCCCGCTCCGCCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACA
GGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGC
TTGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGG
GGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCTCCGC
GCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGTGTGC
GCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAA
GGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCGGTCGGGCT
GCAACCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGC
TCCGTACGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTG
CCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCG
GAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCG
AGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGC
CGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGG
GGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTC
CGCGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTG
ACCGGCGGC

Fig. 32

Nucleotide sequence of 2IRES insert region

(1) (2)
Sal1-EcoR1-BglII-KpnI-SpeI-Bip IRES-BamH1-HindIII-NheI-Myc IRES-XhoI-StuI-XbaI-BssHII

ACGCGTCGACGTCGGCCATACCGGAATTCCGGGGAAGATCTTCCCGGGGTACCCCGAGGACT
AGTtcgacgccggccaagacagcacagacagattgacctattggggtgtttcgcgagtgtga (1)
gagggaagcgccgcggcctgtattactagacctgcccttcgcctggttcgtggcgccttgtg
accccgggcccctgccgcctgcaagtcgaaattgcgctgtgctcctgtgctacggcctgtgg
ctggactgcctgctgctgccctactggctggcaagatcaagctctccctggtggccgcgatc
ctCGCGGATCCGCGCCCAAGCTTGGGTTAGCTAGCccctaattccagcgagaggcagaggga (2)
gcgagcgggcggccggctagggtggaagagccgggcgagcagagctgcgctgcgggcgtcct
gggaagggagatccggagcgaatagggggcttcgcctctggcccagccctcccgctgatccc
ccagccagcggtccgcaacccttgccgcatccacgaaactttgcccatagcagcgggcgggc
actttgcactggaacttacaacacccgagcaaggacgcgactctcccgacgcggggaggcta
ttctgcccatttggggacacttccccgccgctgccaggacccgcttctctgaaaggctctcc
ttgcagctgcttagacgctggatttttttcgggtagtggaaaaccagcagcctcccgcgCCG
CTCGAGCGGAAAAGGCCTTTTGCTCTAGAGCTTGGCGCGCCAA

Fig. 33

Nucleotide sequence of SV40ori-UAS-CMVi-RU5' region

(1) (2) (3)
SalI-SV40 ori + **UAS** + CMVi + **RU5'**-EcoR1

GTCGACGTCGGCCATA
aatttttgcaaaagccttggcctccaaaaaagcctcctcactacttctggaatagctcaga (1)
ggccgaggcggcctcggcctctgcataaataaaaaaaattagtcagccttggggcggagaaa
ctatcgttgctgactaattgagat**cggagtactgtcctccg**cgttacataacttacggtaaa (2)
tggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttc
ccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaact
gcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatga
cggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggc
agtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatcaat
gggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaatgg
gagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgccccat
tgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctcgtttagtg
aaccgtcagatcgcctggagacgccatccacgctgttttgacctccatagaagacaccggga
ccgatccagcctccgcggccgggaacggtgcattggaacgcggattccccgtgccaagagtg
acgtaagtaccgcctatagactctataggcacacccctttggctcttatccatcaattaata
cgactcactatagggagacagactgttcctttcctgggtcttttctg**gcttcgaggggctcg** (3)
catctctccttcacgcgcccgccgccctacctgaggccgccatccacgccggttgagtcgcg
ttctgccgcctcccgcctgtggtgcctcctgaactgcgtccgccgtctaggtaagtttaaag
ctcaggtcgagaccgggcctttgtccggcgctcccttggagcctacctagactcagccggct
ctccacgctttgcctgaccctgcttgctcaactctacgtctttgtttcgttttctgttctgc
gccgttacagatccaagccacc CCGGAATTC

Fig. 34

Nucleotide sequence of 3 x stop-BGH-polyA-UAS-hTERT enhancer + SV40 enhancer + CMV enhancer region

(1) (2) (3) (4)
Xba1-3xstop-BGHpA-**UAS**-hTERT enh-SV40 enh.-CMV enh.-BssHII

TCTAGAGC*tagatgactaac*GTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGC (1)
CAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCAC
TGTCCTTTCCT*AATAAAAT*GAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTC
TGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCT
GGGGATGCGGTGGGCTCTATGG**cggagtactgtcctccg**cttcccacgtggcggagggactg (2)
gggacccgggcacccgtcctgccccttcaccttccagctccgcctcctccgcgcggaccccg
ccccgtcccgaccccctcccgggtccccggcccagcccccctccgggccctcccagccccctccc
cttcctttccgcggccccgccctctcctcgcggcgcgagtttTGGAAAGTCCCCAGGCTCCC (3)
CAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCC
CCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAACCATAGT
CCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCC
ATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTC
CAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCAAGGCTTTTGCAAAAAGCTCcgttacataac (4)
ttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatg
acgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtattt
acggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgcccccctattg
acgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggacttt
cctacttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggca
gtacatcaatgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattg
acgtcaatgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaac
tccgccccattgacgcaaatgggcggtaggcgtgTTGGCGCGC

Fig. 35-1

Full nucleotide sequence of construct No. 14 vector

GGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTC
TTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATT
AGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTA
CACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTCGGAAAAAGAGT
TGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGC
AGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATTTTCTACCGAAGAAAG
GCCCACCCGTGAAGGTGAGCCAGTGAGTTGATTGCAGTCCAGTTACGCTGGAGTCTGAGGCT
CGTCCTGAATGTGTAAAACGACGGCCAGTTTATCTAGTCAGCTTGATTCTAGCTGATCGTGG
ACCGGAAGGTGAGCCAGTGAGTTGATTGCAGTCCAGTTACGCTGGAGTCTGAGGCTCGTCCT
GAATGATAT**ACGCGTCGGAGGGTTGCGTTTGAGACGGGCGACAGAT**ACGCGTCGACGTCGGC
CATAaatttttgcaaaagccttggcctccaaaaaagcctcctcactacttctggaatagct (1)
cagaggccgaggcggcctcggcctctgcataaataaaaaaaattagtcagccttggggcgga
gaaactatcgttgctgactaattgagat**cggagtactgtcctccg**cgttacataacttacgg (2)
taaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtat
gttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggta
aactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtca
atgacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctact
tggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacat
caatgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtca
atgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgcc
ccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctcgttt
agtgaaccgtcagatcgcctggagacgccatccacgctgttttgacctccatagaagacacc
gggaccgatccagcctccgcggccgggaacggtgcattggaacgcggattccccgtgccaag
agtgacgtaagtaccgcctatagactctataggcacacccctttggctcttatccatcaatt
aatacgactcactatagggagacagactgttcctttcctgggtcttttctg**gcttcgagggg** (3)
ctcgcatctctccttcacgcgcccgccgccctacctgaggccgccatccacgccggttgagt
cgcgttctgccgcctcccgcctgtggtgcctcctgaactgcgtccgccgtctaggtaagttt
aaagctcaggtcgagaccgggcctttgtccggcgctcccttggagcctacctagactcagcc
ggctctccacgctttgcctgaccctgcttgctcaactctacgtctttgtttcgttttctgtt
ctgcgccgttacagatccaagccaccCCGGAATTCCGGGGAAGATCTTCCCGGGGTACCCCG
AGGACTAGTtcgacgccggccaagacagcacagacagattgacctattggggtgtttcgcga (4)
gtgtgagagggaagcgccgcggcctgtattactagacctgcccttcgcctggttcgtggcgc
cttgtgaccccgggcccctgccgcctgcaagtcgaaattgcgctgtgctcctgtgctacggc
ctgtggctggactgcctgctgctgccctactggctggcaagatcaagctctccctggtggcc
gcgatcctCGCGGATCCGCGCCCAAGCTTGGGTTAGCTAGCccctaattccagcgagaggca (5)
gagggagcgagcgggcggccggctagggtggaagagccgggcgagcagagctgcgctgcggg
cgtcctgggaagggagatccggagcgaatagggggcttcgcctctggcccagccctcccgct
gatccccagccagcggtccgcaacccttgccgcatccacgaaactttgcccatagcagcgg
gcgggcactttgcactggaacttacaacacccgagcaaggacgcgactctcccgacgcgggg
aggctattctgcccatttggggacacttccccgccgctgccaggacccgcttctctgaaagg
ctctccttgcagctgcttagacgctggatttttttcgggtagtggaaaaccagcagcctccc
gcgCCGCTCGAGCGGAAAAGGCCTTTTGCTCTAGAGCtagatgactaacGTTTAAACCCGCT

Fig. 35-2

GATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCT
TCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATC
GCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGG
AGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG**cggagtactgt**
cctccgcttcccacgtggcggagggactggggacccgggcacccgtcctgcccttcacctt (6)
ccagctccgcctcctccgcgcggaccccgccccgtcccgacccctccgggtccccggccca
gccccctccgggccctcccagccccctccccttcctttccgcggccccgccctctcctcgcgg
cgcgagtttTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCA (7)
ATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGC
ATCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAAC
TCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGG
CCGAGGCCGCCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCAA
GGCTTTTGCAAAAAGCTCcgttacataacttacggtaaatggcccgcctggctgaccgccca
acgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggact
ttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggcagtacatcaagt
gtatcatatgccaagtacgccccctattgacgtcaatgacggtaaatggcccgcctggcatt
atgcccagtacatgaccttatgggactttcctacttggcagtacatctacgtattagtcatc
gctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatagcggtttgactc
acggggatttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatc
aacgggactttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgt
gTTGGCGCGCCAA**ATCAGTTCTGGACGAGCGAGCTGTCGTCCGGCGGCCGC**GATCTTACGGC
ATTATACGTATGATCGGTCCACGATCAGCTAGATTATCTAGTCAGCTTGATGTCATAGCTGT
TTCCTGAGGCTCAATACTGACCATTTAAATCATACCTGACCTCCATAGCAGAAAGTCAAAAG
CCTCCGACCGGAGGCTTTTGACTTGATCGGCACGTAAGAGGTTCCAACTTTCACCATAATGA
AATAAGATCACTACCGGGCGTATTTTTTGAGTTATCGAGATTTTCAGGAGCTAAGGAAGCTA
AAATGAGCCATATTCAACGGGAAACGTCTTGCTTGAAGCCGCGATTAAATTCCAACATGGAT
GCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTA
TCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTG
CCAATGATGTTACAGATGAGATGGTCAGGCTAAACTGGCTGACGGAATTTATGCCTCTTCCG
ACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCAGG
GAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGC
TGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACGGCGAT
CGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGGTGCGAGTGA
TTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTCT
TGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTT
GACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCA
GGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTT
TTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCACTTGATGCTCGAT
GAGTTTTTCTAATGAGGACCTAAATGTAATCACCTGGCTCACCTTCGGGTGGGCCTTTCTGC
GTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGATGCTCAA
GTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCC
CTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTC
GGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTC
GCTCCAAGCT

Fig. 36

Construct No.15

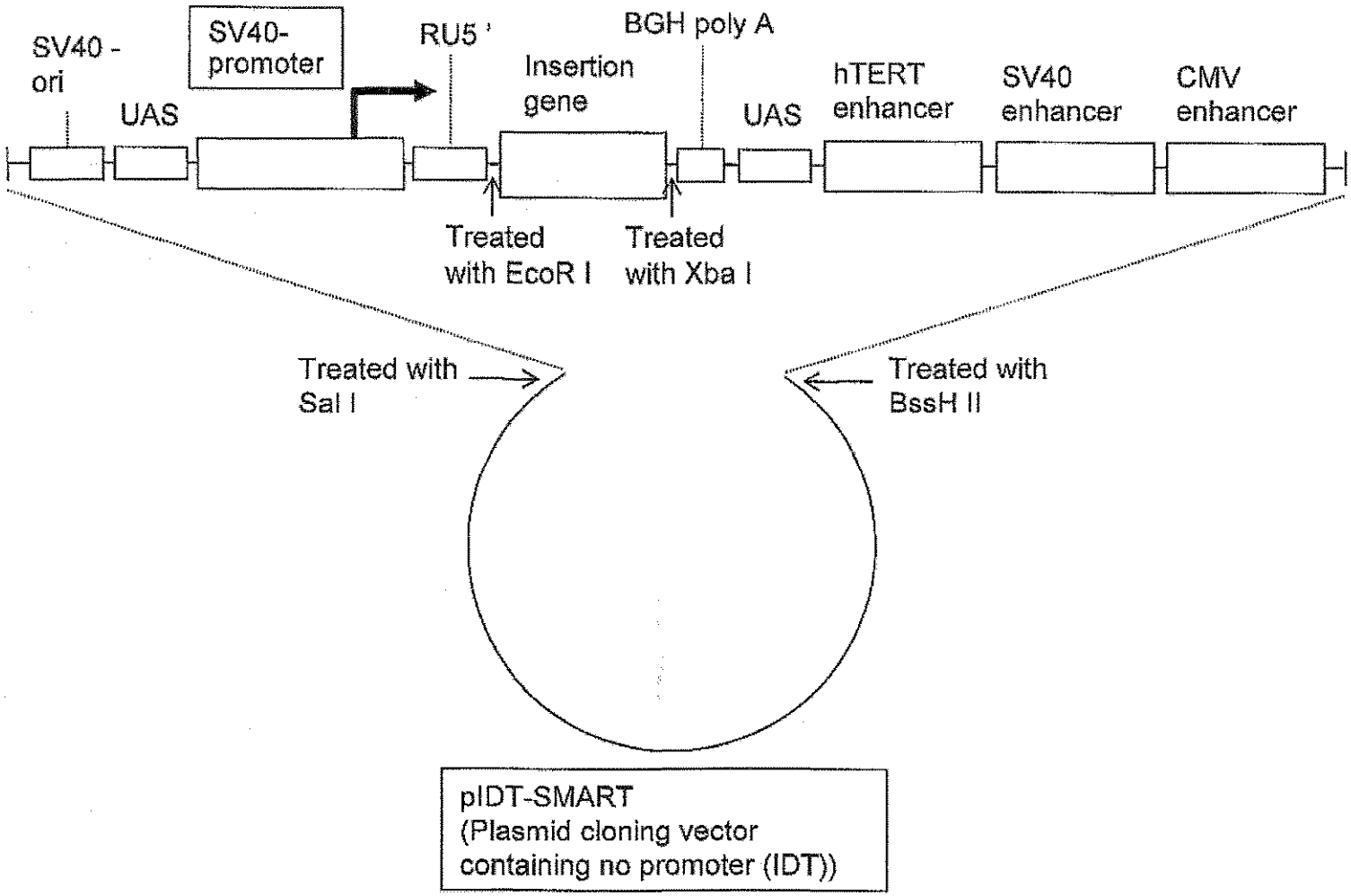


Fig. 37

Construct No.16

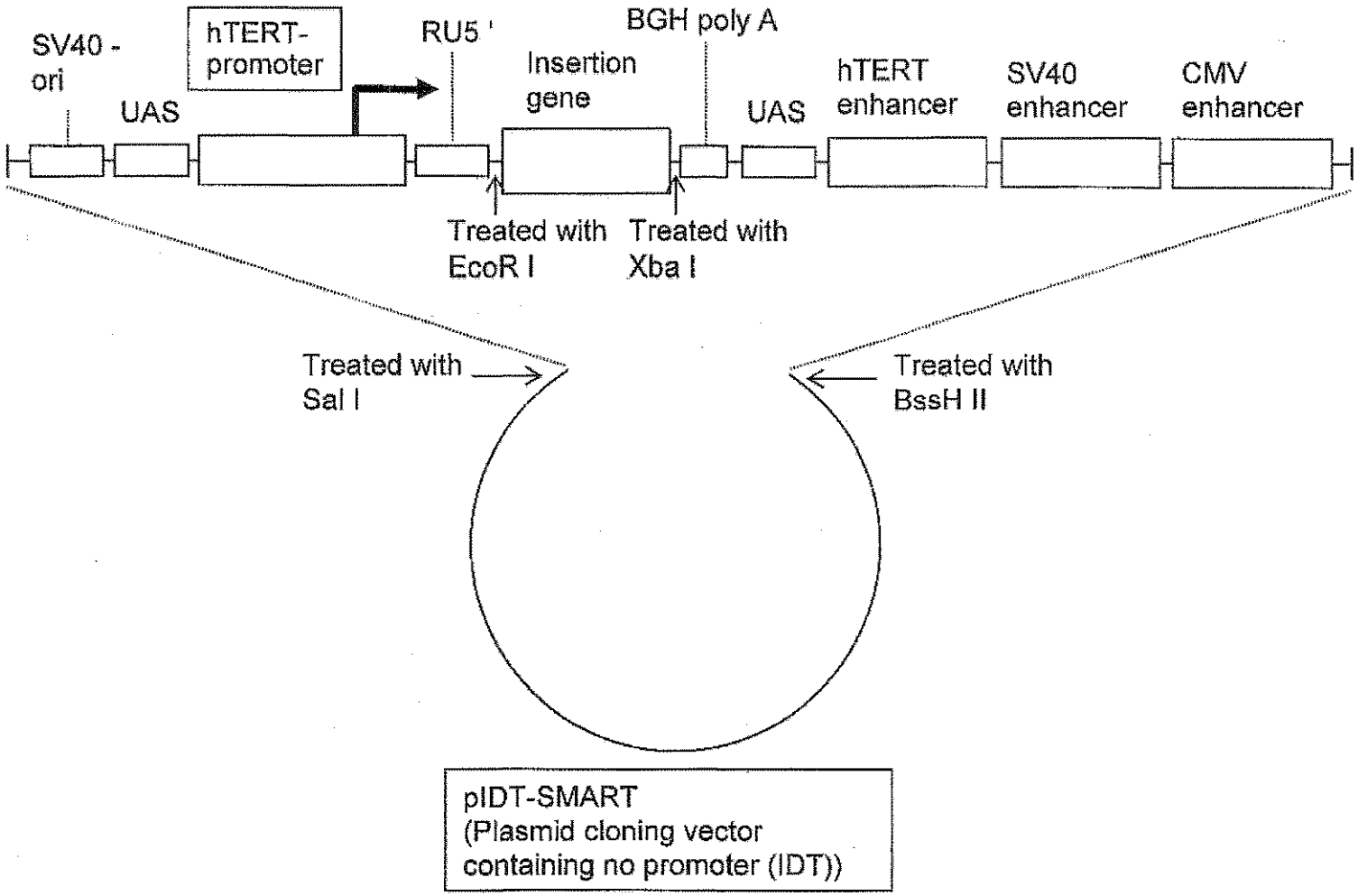


Fig. 38

P-SViRU: 1072 bp ---- pIDT SMART (Kan)

(1) (2) (3) (4)
SalI-SV40 ori+**UAS**+SV40 enh+intron A+RU5'-EcoR1

ACGCG/TCGACGTCGGCCATA
aatttttgcaaaagccttggcctccaaaaaagcctcctcactacttctggaatagctcaga (1)
ggccgaggcggcctcggcctctgcataaataaaaaaaattagtcagccttggggcggagaaa
ctatcgttgctgactaattgagat **cggagtactgtcctccg**
TGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAG (2)
CAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTC
AATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAG
TTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCG
CCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGC
AAAAAGCTC
tacggtgggaggtctatataagcagagctcgtttagtgaaccgtcagatcgcctggagacgc (3)
catccacgctgttttgacctccatagaagacaccgggaccgatccagcctccgcggccgggga
acggtgcattggaacgcggattccccgtgccaagagtgacgtaagtaccgcctatagactct
ataggcacacccctttggctcttatccatcaattaatacgactcactatagggagacagact
gttcctttcctgggtcttttctg
gcttcgaggggctcgcatctctccttcacgcgcccgccgccctacctgaggccgccatccac (4)
gccggttgagtcgcgttctgccgcctcccgcctgtggtgcctcctgaactgcgtccgccgtc
taggtaagtttaaagctcaggtcgagaccgggcctttgtccggcgctcccttggagcctacc
tagactcagccggctctccacgctttgcctgaccctgcttgctcaactctacgtctttgttt
cgttttctgttctgcgccgttacagatccaagccacc CCGG/AATTCCGG

Fig. 39

P-TiRU: 942 bp ---- pIDT SMART (Kan)

(1) (2) (3) (4)
SalI-SV40 ori+**UAS**+hTERT enh+intron A+RU5'-EcoR1

ACGCG/TCGACGTCGGCCATA
aatttttgcaaaagccttggcctccaaaaaagcctcctcactacttctggaatagctcaga (1)
ggccgaggcggcctcggcctctgcataaataaaaaaaattagtcagccttggggcggagaaa
ctatcgttgctgactaattgagat **cggagtactgtcctccg**
cttcccacgtggcggagggactggggacccgggcacccgtcctgccccttcaccttccagct (2)
ccgcctcctccgcgcggaccccgccccgtcccgaccccctcccgggtccccggcccagccccc
tccgggccctcccagccccctcccttcctttccgcggccccgccctctcctcgcggcgcgag
ttt
tacggtgggaggtctatataagcagagctcgtttagtgaaccgtcagatcgcctggagacgc (3)
catccacgctgttttgacctccatagaagacaccgggaccgatccagcctccgcggccgggga
acggtgcattggaacgcggattccccgtgccaagagtgacgtaagtaccgcctatagactct
ataggcacacccctttggctcttatccatcaattaatacgactcactatagggagacagact
gttcctttcctgggtcttttctg
gcttcgaggggctcgcatctctccttcacgcgcccgccgccctacctgaggccgccatccac (4)
gccggttgagtcgcgttctgccgcctcccgcctgtggtgcctcctgaactgcgtccgccgtc
taggtaagtttaaagctcaggtcgagaccgggcctttgtccggcgctcccttggagcctacc
tagactcagccggctctccacgctttgcctgaccctgcttgctcaactctacgtctttgttt
cgttttctgttctgcgccgttacagatccaagccacc CCGG/AATTCCGG

Fig. 40

GFP (EcoR1-BamH1)

CCGG/AATTCCGG acc atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg
gtg ccc atc ctg gtc gag ctg gac ggc gac gta aac ggc cac aag ttc
agc gtg tcc ggc gag ggc gag ggc gat gcc acc tac ggc aag ctg acc
ctg aag ttc atc tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc
ctc gtg acc acc ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc
gac cac atg aag cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc
tac gtc cag gag cgc acc atc ttc ttc aag gac gac ggc aac tac aag
acc cgc gcc gag gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc
gag ctg aag ggc atc gac ttc aag gag gac ggc aac atc ctg ggg cac
aag ctg gag tac aac tac aac agc cac aac gtc tat atc atg gcc gac
aag cag aag aac ggc atc aag gtg aac ttc aag atc cgc cac aac atc
gag gac ggc agc gtg cag ctc gcc gac cac tac cag cag aac acc ccc
atc ggc gac ggc ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc
cag tcc gcc ctg agc aaa gac ccc aac gag aag cgc gat cac atg gtc
ctg ctg gag ttc gtg acc gcc gcc ggg atc act ctc ggc atg gac gag
ctg tac aag tga CGCG/GATCCGCG

Fig. 41-1

#14-GFP

(1) (2) (3)
SalI- **P-CMViRU** -EcoRI- **GFP** -BamHI-HindIII-NheI-Myc IRES-XhoI-

(4)
StuI-XbaI- **pA-3enh** -BssHII

ACGCGTCGACGTCGGCCATA

aatttttgcaaaagccttggcctccaaaaaagcctcctcactacttctggaatagctcaga (1)
ggccgaggcggcctcggcctctgcataaataaaaaaaattagtcagccttggggcggagaaa
ctatcgttgctgactaattgagatcggagtactgtcctccgcgttacataacttacggtaaa
tggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttc
ccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaact
gcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatga
cggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggc
agtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatcaat
gggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaatgg
gagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgccccat
tgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctcgtttagtg
aaccgtcagatcgcctggagacgccatccacgctgttttgacctccatagaagacaccggga
ccgatccagcctccgcggccgggaacggtgcattggaacgcggattccccgtgccaagagtg
acgtaagtaccgcctatagactctataggcacacccctttggctcttatccatcaattaata
cgactcactatagggagacagactgttcctttcctgggtcttttctggcttcgaggggctcg
catctctccttcacgcgcccgccgccctacctgaggccgccatccacgccggttgagtcgcg
ttctgccgcctcccgcctgtggtgcctcctgaactgcgtccgccgtctaggtaagtttaaag
ctcaggtcgagaccgggcctttgtccggcgctcccttggagcctacctagactcagccggct
ctccacgctttgcctgaccctgcttgctcaactctacgtctttgtttcgttttctgttctgc
gccgttacagatccaagccacc CCGGAATTCCGG acc atg gtg agc aag ggc gag (2)
gag ctg ttc acc ggg gtg gtg ccc atc ctg gtc gag ctg gac ggc gac
gta aac ggc cac aag ttc agc gtg tcc ggc gag ggc gag ggc gat gcc
acc tac ggc aag ctg acc ctg aag ttc atc tgc acc acc ggc aag ctg
ccc gtg ccc tgg ccc acc ctc gtg acc acc ctg acc tac ggc gtg cag
tgc ttc agc cgc tac ccc gac cac atg aag cag cac gac ttc ttc aag
tcc gcc atg ccc gaa ggc tac gtc cag gag cgc acc atc ttc ttc aag
gac gac ggc aac tac aag acc cgc gcc gag gtg aag ttc gag ggc gac
acc ctg gtg aac cgc atc gag ctg aag ggc atc gac ttc aag gag gac
ggc aac atc ctg ggg cac aag ctg gag tac aac tac aac agc cac aac
gtc tat atc atg gcc gac aag cag aag aac ggc atc aag gtg aac ttc
aag atc cgc cac aac atc gag gac ggc agc gtg cag ctc gcc gac cac
tac cag cag aac acc ccc atc ggc gac ggc ccc gtg ctg ctg ccc gac
aac cac tac ctg agc acc cag tcc gcc ctg agc aaa gac ccc aac gag
aag cgc gat cac atg gtc ctg ctg gag ttc gtg acc gcc gcc ggg atc
act ctc ggc atg gac gag ctg tac aag tga CGCGGATCCGCG CCCAAGCTTGGG
TTAGCTAGC

Fig. 41-2


ccctaattccagcgagaggcagagggagcgagcgggcggccggctagggtggaagagccggg (3)
cgagcagagctgcgctgcgggcgtcctgggaagggagatccggagcgaatagggggcttcgc
ctctggcccagccctcccgctgatcccccagccagcggtccgcaacccttgccgcatccacg
aaactttgcccatagcagcgggcgggcactttgcactggaacttacaacacccgagcaagga
cgcgactctcccgacgcggggaggctattctgcccatttggggacacttccccgccgctgcc
aggacccgcttctctgaaaggctctccttgcagctgcttagacgctggatttttttcgggta
gtggaaaaccagcagcctcccgcg CCGCTCGAGCGG AAAAGGCCTTTT GCTCTAGAGC
tagatgactaacGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATC (4)
TGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTT
CCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGT
GGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGC
GGTGGGCTCTATGGcggagtactgtcctccgcttcccacgtggcggagggactggggacccg
ggcacccgtcctgccccttcaccttccagctccgcctcctccgcgcggaccccgccccgtcc
cgaccctcccgggtccccggcccagccccctccgggccctcccagccccctcccccttcctttt
ccgcggccccgccctctcctcgcggcgcgagtttTGGAAAGTCCCCAGGCTCCCCAGCAGGC
AGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTC
CCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCC
TAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA
CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTCCAGAAGTA
GTGAGGAGGCTTTTTTGGAGGCCAAGGCTTTTGCAAAAAGCTCcgttacataacttacggta
aatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgt
tcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaa
ctgcccacttggcagtacatcaagtgtatcatatgccaagtacgcccccctattgacgtcaat
gacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttg
gcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatca
atgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaat
gggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgcccc
attgacgcaaatgggcggtaggcgtg TTGGCGCGCCAA

Fig. 42-1

#15-GFP

(1) (2) (3)

Sal1- **P-SViRU** -EcoR1- **GFP** -BamH1-HindIII-NheI-Myc IRES-XhoI-

(4)

StuI-XbaI- **pA-3enh** -BssHII

ACGCGTCGACGTCGGCCATA

aatttttttgcaaaagccttggcctccaaaaaagcctcctcactacttctggaatagctcaga (1)
ggccgaggcggcctcggcctctgcataaataaaaaaaattagtcagccttggggcggagaaa
ctatcgttgctgactaattgagatcggagtactgtcctccgTGGAAAGTCCCCAGGCTCCCC
AGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCC
CAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTC
CCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCA
TGGCTGACTAATTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTCC
AGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTCtacggtgggagg
tctatataagcagagctcgtttagtgaaccgtcagatcgcctggagacgccatccacgctgt
tttgacctccatagaagacaccgggaccgatccagcctccgcggccgggaacggtgcattgg
aacgcggattccccgtgccaagagtgacgtaagtaccgcctatagactctataggcacaccc
ctttggctcttatccatcaattaatacgactcactatagggagacagactgttcctttcctg
ggtcttttctggcttcgaggggctcgcatctctccttcacgcgcccgccgccctacctgagg
ccgccatccacgccggttgagtcgcgttctgccgcctcccgcctgtggtgcctcctgaactg
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tggagcctacctagactcagccggctctccacgctttgcctgaccctgcttgctcaactcta
cgtctttgtttcgttttctgttctgcgccgttacagatccaagccacc CCGGAATTCCGG

acc atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc (2)
ctg gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc
ggc gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc
atc tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc
acc ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg
aag cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag
gag cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc
gag gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag
ggc atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag
tac aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag
aac ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc
agc gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac
ggc ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc
ctg agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag
ttc gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag
tga CGCGGATCCGCG

Fig. 42-2

CCCAAGCTTGGG TTAGCTAGC

ccctaattccagcgagaggcagagggagcgagcgggcggccggctagggtggaagagccggg (3)
cgagcagagctgcgctgcgggcgtcctgggaagggagatccggagcgaatagggggcttcgc
ctctggcccagccctcccgctgatcccccagccagcggtccgcaacccttgccgcatccacg
aaactttgcccatagcagcgggcgggcactttgcactggaacttacaacacccgagcaagga
cgcgactctcccgacgcggggaggctattctgcccatttggggacacttcccccgccgctgcc
aggacccgcttctctgaaaggctctccttgcagctgcttagacgctggatttttttcgggta
gtggaaaaccagcagcctcccgcg CCGCTCGAGCGG AAAAGGCCTTTT GCTCTAGAGC

tagatgactaacGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATC (4)
TGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTT
CCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGT
GGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGC
GGTGGGCTCTATGGcggagtactgtcctccgcttcccacgtggcggagggactggggacccg
ggcacccgtcctgcccctcacctccagctccgcctcctccgcgcggaccccgccccgtcc
cgacccctcccgggtccccggcccagccccctccgggccctcccagccccctccccttcctttt
ccgcggccccgccctctcctcgcggcgcgagtttTGGAAAGTCCCCAGGCTCCCCAGCAGGC
AGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTC
CCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCC
TAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA
CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTCCAGAAGTA
GTGAGGAGGCTTTTTTGGAGGCCAAGGCTTTTGCAAAAAGCTCcgttacataacttacggta
aatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgt
tcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaa
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gacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttg
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atgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaat
gggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgcccc
attgacgcaaatgggcggtaggcgtg TTGGCGCGCCAA

Fig. 43-1

#16-GFP

(1) (2) (3)
Sal1- **P-TiRU** -EcoR1- **GFP** -BamH1-HindIII-NheI-Myc IRES-XhoI-

(4)
StuI-XbaI- **pA-3enh** -BssHII

ACGCGTCGACGTCGGCCATA

aatttttttgcaaaagccttggcctccaaaaaagcctcctcactacttctggaatagctcaga (1)
ggccgaggcggcctcggcctctgcataaataaaaaaaattagtcagccttggggcggagaaa
ctatcgttgctgactaattgagatcggagtactgtcctccgcttcccacgtggcggagggac
tggggaccccgggcacccgtcctgcccccttcaccttccagctccgcctcctccgcgcggaccc
cgccccgtcccgacccctcccgggtccccggcccagcccccctccgggccctcccagccccctc
ccctttcctttccgcggccccgccctctcctcgcggcgcgagttttacggtgggaggtctata
taagcagagctcgtttagtgaaccgtcagatcgcctggagacgccatccacgctgttttgac
ctccatagaagacaccgggaccgatccagcctccgcggccgggaacggtgcattggaacgcg
gattccccgtgccaagagtgacgtaagtaccgcctatagactctataggcacacccctttgg
ctcttatccatcaattaatacgactcactatagggagacagactgttcctttcctgggtctt
ttctggcttcgaggggctcgcatctctccttcacgcgcccgccgccctacctgaggccgcca
tccacgccggttgagtcgcgttctgccgcctcccgcctgtggtgcctcctgaactgcgtccg
ccgtctaggtaagtttaaagctcaggtcgagaccgggcctttgtccggcgctcccttggagc
ctacctagactcagccggctctccacgctttgcctgaccctgcttgctcaactctacgtctt
tgtttcgttttctgttctgcgccgttacagatccaagccacc CCGGAATTCCGG acc atg (2)
gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg gtc
gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc gag
ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc tgc
acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc ctg
acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag cag
cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag cgc
acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag gtg
aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc atc
gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac aac
tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac ggc
atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc gtg
cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc ccc
gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg agc
aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc gtg
acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag tga

CGCGGATCCGCG

Fig. 43-2

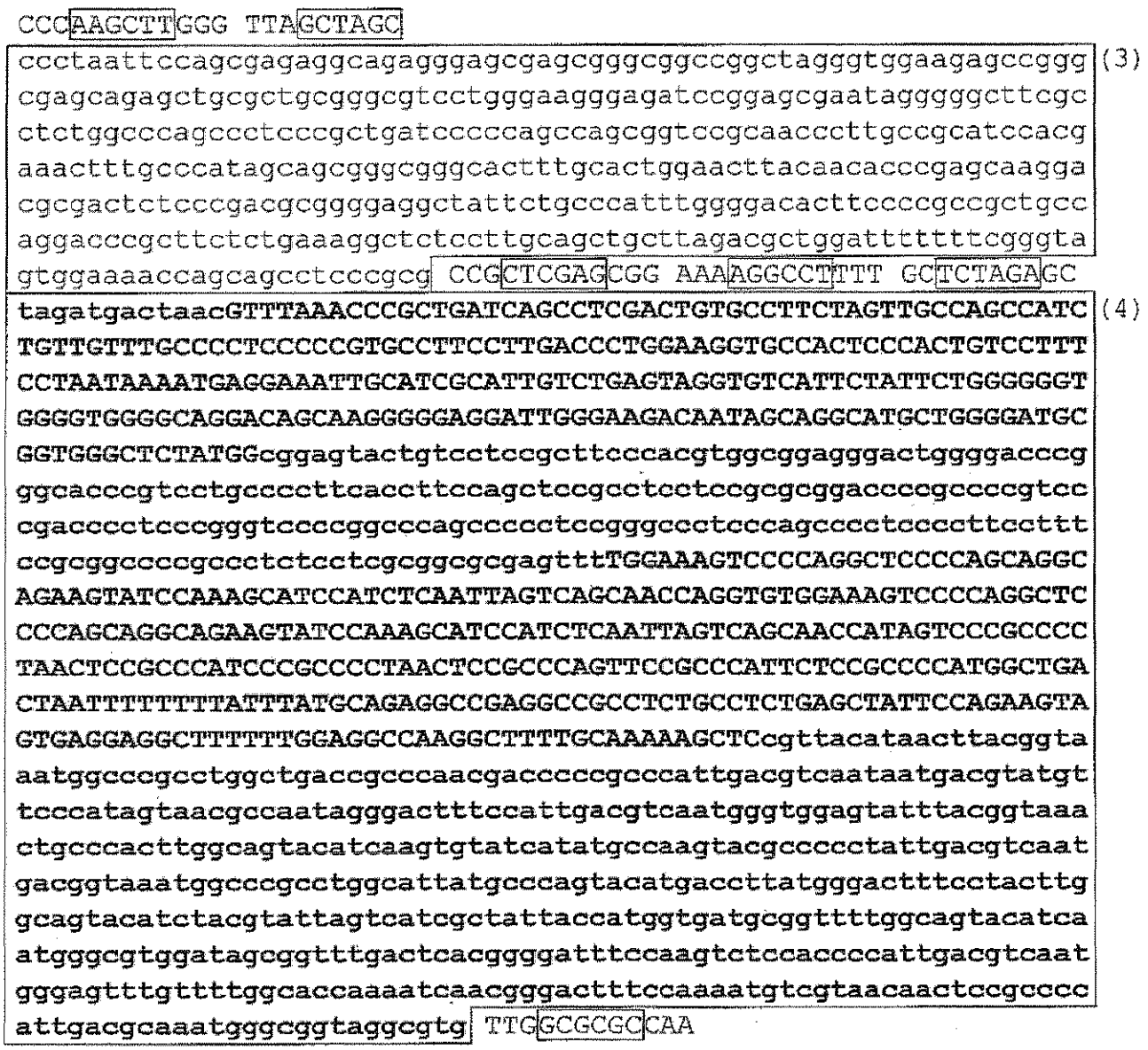

CCCAAGCTTGGG TTAGCTAGC
ccctaattccagcgagaggcagagggagcgagcgggcggccggctagggtggaagagccggg (3)
cgagcagagctgcgctgcgggcgtcctgggaagggagatccggagcgaatagggggcttcgc
ctctggcccagccctcccgctgatcccccagccagcggtccgcaacccttgccgcatccacg
aaactttgcccatagcagcgggcgggcactttgcactggaacttacaacacccgagcaagga
cgcgactctcccgacgcggggaggctattctgcccatttggggacacttccccgccgctgcc
aggacccgcttctctgaaaggctcttcttgcagctgcttagacgctggatttttttcgggta
gtggaaaaccagcagcctcccgcg CCGCTCGAGCGG AAAAGGCCTTTT GCTCTAGAGC
tagatgactaacGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATC (4)
TGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTT
CCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGT
GGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGC
GGTGGGCTCTATGGcggagtactgtcctccgcttcccacgtggcggagggactggggacccg
ggcacccgtcctgcccctcaccttccagctccgcctcctccgcgcggaccccgccccgtcc
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AGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTC
CCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCC
TAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA
CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTCCAGAAGTA
GTGAGGAGGCTTTTTTGGAGGCCAAGGCTTTTGCAAAAAGCTCcgttacataacttacggta
aatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgt
tcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaa
ctgcccacttggcagtacatcaagtgtatcatatgccaagtacgcccccctattgacgtcaat
gacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttg
gcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatca
atgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaat
gggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgcccc
attgacgcaaatgggcggtaggcgtg TTGGCGCGCCAA

Fig. 44

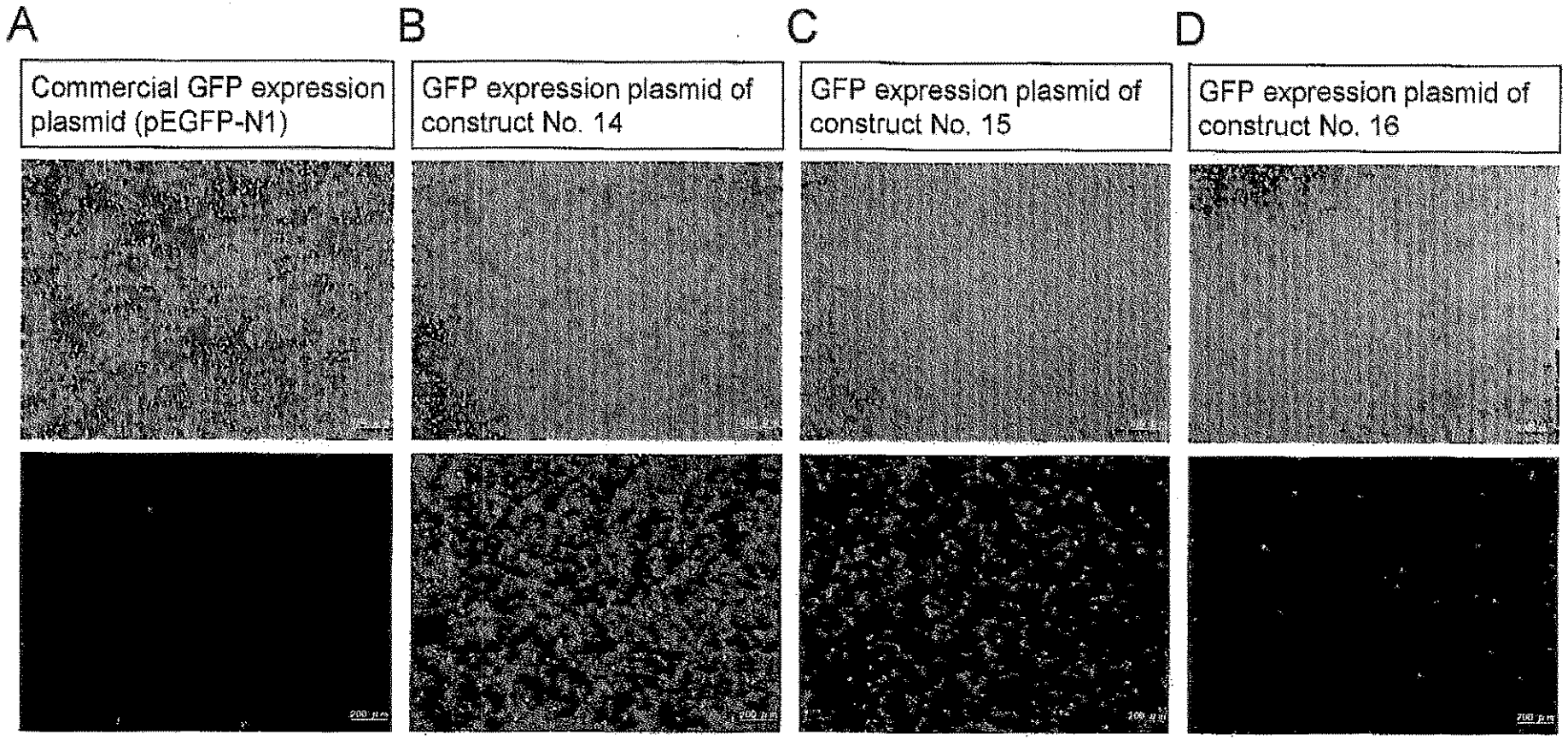

A
B
C
D
Commercial GFP expression plasmid (pEGFP-N1)
GFP expression plasmid of construct No. 14
GFP expression plasmid of construct No. 15
GFP expression plasmid of construct No. 16

Fig. 45

EPO-6His gene sequence

CCGG/AATTCCGGaccatggggtgcacgaatgtcctgcctggctgtggcttctcctgtccc
tgctgtcgctccctctgggcctcccagtcctgggcgccccaccacgcctcatctgtgacagc
cgagtcctggagaggtacctcttggaggccaaggaggccgagaatatcacgacgggctgtgc
tgaacactgcagcttgaatgagaatatcactgtcccagacaccaaagttaatttctatgcct
ggaagaggatggaggtcgggcagcaggccgtagaagtctggcagggcctggccctgctgtcg
gaagctgtcctgcggggccaggccctgttggtcaactcttcccagccgtgggagcccctgca
gctgcatgtggataaagccgtcagtggccttcgcagcctcaccactctgcttcgggctctgg
gagcccagaaggaagccatctcccctccagatgcggcctcagctgctccactccgaacaatc
actgctgacactttccgcaaactcttccgagtctactccaatttcctccggggaaagctgaa
gctgtacacaggggaggcctgcaggacaggggacaga gga cca ggt **cat cac cac cat**
cac cat tga**GCT/CTAGAGC**

Fig. 46

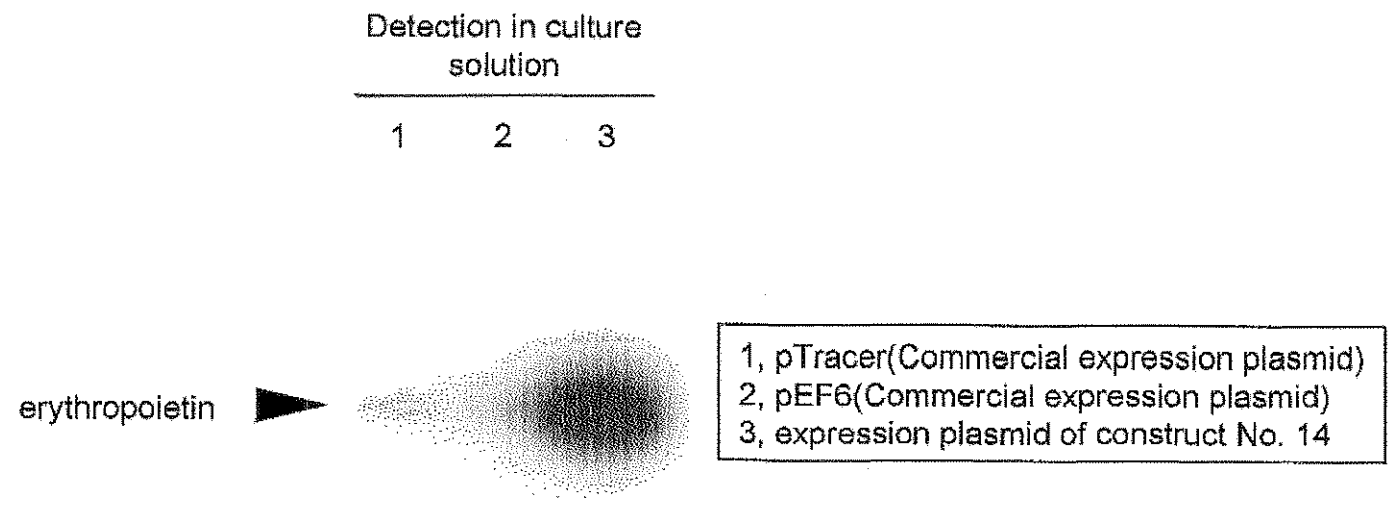

Detection in culture solution
1
2
3
erythropoietin
1, pTracer(Commercial expression plasmid)
2, pEF6(Commercial expression plasmid)
3, expression plasmid of construct No. 14

Fig. 47A

Human IgG (light chain) gene sequence

(1) (2)
EcoRI - XhoI - Light chain (Myc ab/ clone 9E10)-6His - XbaI

G/AATTCCGG CCGCTCGAGCGG acc atg gag aaa gac aca ctc ctg cta tgg (1)
gtc ctg ctt ctc tgg gtt cca ggt tcc aca ggt gac att gtg ctg acc
caa tct cca gct tct ttg gct gta tct cta gga cag agg gcc acc atc
tcc tgc aga gcc agc gaa agt gtt gat aat tat ggc ttt agt ttt atg
aac tgg ttc cag cag aaa cca gga cag ccc ccc aaa ctc ctc atc tat
gct ata tcc aac cga ggg tcc ggg gtc cct gcc agg ttt agt ggc agt
ggg tct ggg aca gac ttc agc ctc aac atc cat cct gta gag gag gat
gat cct gca atg tat ttc tgt cag caa act aag gag gtt ccg tgg acg
ttc ggt gga ggc acc aag ctg gaa atc aaa cgg gct gat gct gca cca
act gta tcc atc ttc cca cca tcc agt gag cag tta aca tct gga ggt
gcc tca gtc gtg tgc ttc ttg aac aac ctc tac ccc aaa gac atc aat
gtc aag tgg aag att gat ggc agt gaa cga caa aat ggc gtc ctg aac
agt tgg act gat cag gac agc aaa gac agc acc tac agc atg agc agc
acc ctc acg ttg acc aag gac gag tat gaa cga cat aac agc tat acc
tgt gag gcc act cac aag aca tca act tca ccc att gtc aag agc ttc
aac agg aat gag tgt gga ccg ggc cat cac cac cat cac cat TGA GCT/CTAGAGC
(2)

Fig. 47B

Human IgG (heavy chain) gene sequence

(1) (2)
EcoRI - Heavy chain (Myc ab/ clone 9E10)-6His - Bam H1 - SpeI - XbaI

G/AATTCCGG acc atg ggg aac ttc ggg ctc agc ttg att ttc ctt gcc ctc (1)
att tta aaa ggt gtc cag tgt gag gtg cac ctg gtg gag tct ggg gga
gac tta gtg aag cct gga ggg tcc ctg aaa ctc tcc tgt gca gcc tct
gga ttc act ttc agt cac tat ggc atg tct tgg gtt cgc cag act cca
gac aag agg ctg gag tgg gtc gca acc att ggt agt cgt ggt act tac
acc cac tat cca gac agt gtg aag gga cga ttc acc atc tcc aga gac
aat gac aag aac gcc ctg tac ctg caa atg aac agt ctg aag tct gaa
gac aca gcc atg tat tac tgt gca aga aga agt gaa ttt tat tac tac
ggt aat acc tac tat tac tct gct atg gac tac tgg ggt caa gga gcc
tca gtc acc gtc tcc tca gcc aaa acg aca ccc cca tct gtc tat cca
ctg gcc cct gga tct gct gcc caa act aac tcc atg gtg acc ctg gga
tgc ctg gtc aag ggc tat ttc cct gag cca gtg aca gtg acc tgg aac
tct ggg tcc ctg tcc agc ggt gtg cac acc ttc cca gct gtc ctg cag
tct gac ctc tac act ctg agc agc tca gtg act gtc ccc tcc agc acc
tgg ccc agc gag acc gtc acc tgc aac gtt gcc cac ccg gcc agc agc
acc aag gtg gac aag aaa att gtg ccc agg gat tgt ggt tgt aag cct
tgc ata tgt aca gtc cca gaa gta tca tct gtc ttc atc ttc ccc cca
aag ccc aag gat gtg ctc acc att act ctg act cct aag gtc acg tgt
gtt gtg gta gac atc agc aag gat gat ccc gag gtc cag ttc agc tgg
ttt gta gat gat gtg gag gtg cac aca gct cag acg caa ccc cgg gag
gag cag ttc aac agc act ttc cgc tca gtc agt gaa ctt ccc atc atg
cac cag gac tgg ccc aat ggc aag gag ttc aaa tgc agg gtc aac agt
gca gct ttc cct gcc ccc atc gag aaa acc atc tcc aaa acc aaa ggc
aga ccg aag gct cca cag gtg tac acc att cca cct ccc aag gag cag
atg gcc aag gat aaa gtc agt ctg acc tgc atg ata aca gac ttc ttc
cct gaa gac att act gtg gag tgg cag tgg aat ggg cag cca gcg gag
aac tac aag aac act cag ccc atc atg aac acg aat ggc tct tac ttc
gtc tac agc aag ctc aat gtg cag aag agc aac tgg gag gca gga aat
act ttc acc tgc tct gtg tta cat gag ggc ctg cac aac cac cat act
gag aag agc ctc tcc cac tct cct ggt aaa gga ccg ggc cat cac cac (2)
cat cac cat TGA CGCGGATCCGCG AGGACTAGTCCT GCT/CTAGAGC

Fig. 48

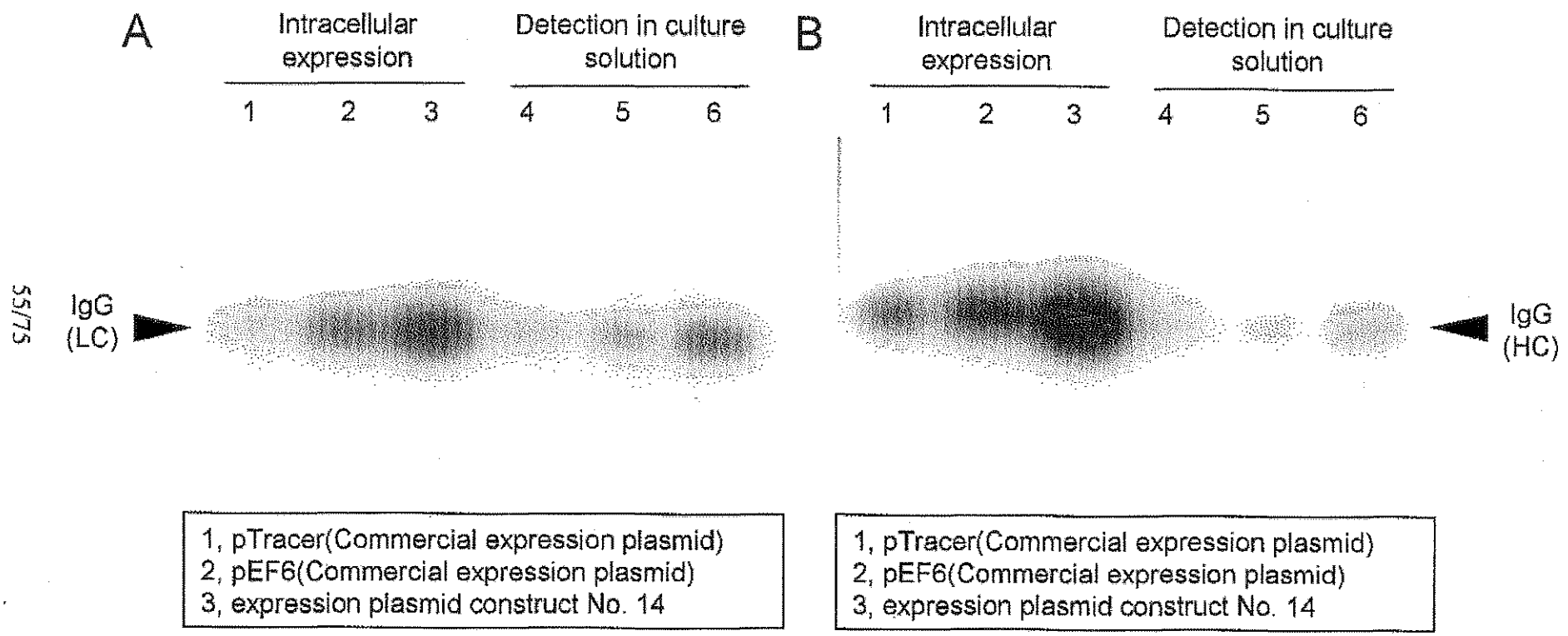

A
Intracellular expression
Detection in culture solution
1 2 3 4 5 6
IgG (LC)
1, pTracer(Commercial expression plasmid)
2, pEF6(Commercial expression plasmid)
3, expression plasmid construct No. 14
B
Intracellular expression
Detection in culture solution
1 2 3 4 5 6
IgG (HC)
1, pTracer(Commercial expression plasmid)
2, pEF6(Commercial expression plasmid)
3, expression plasmid construct No. 14

Fig. 49

Construct No.17

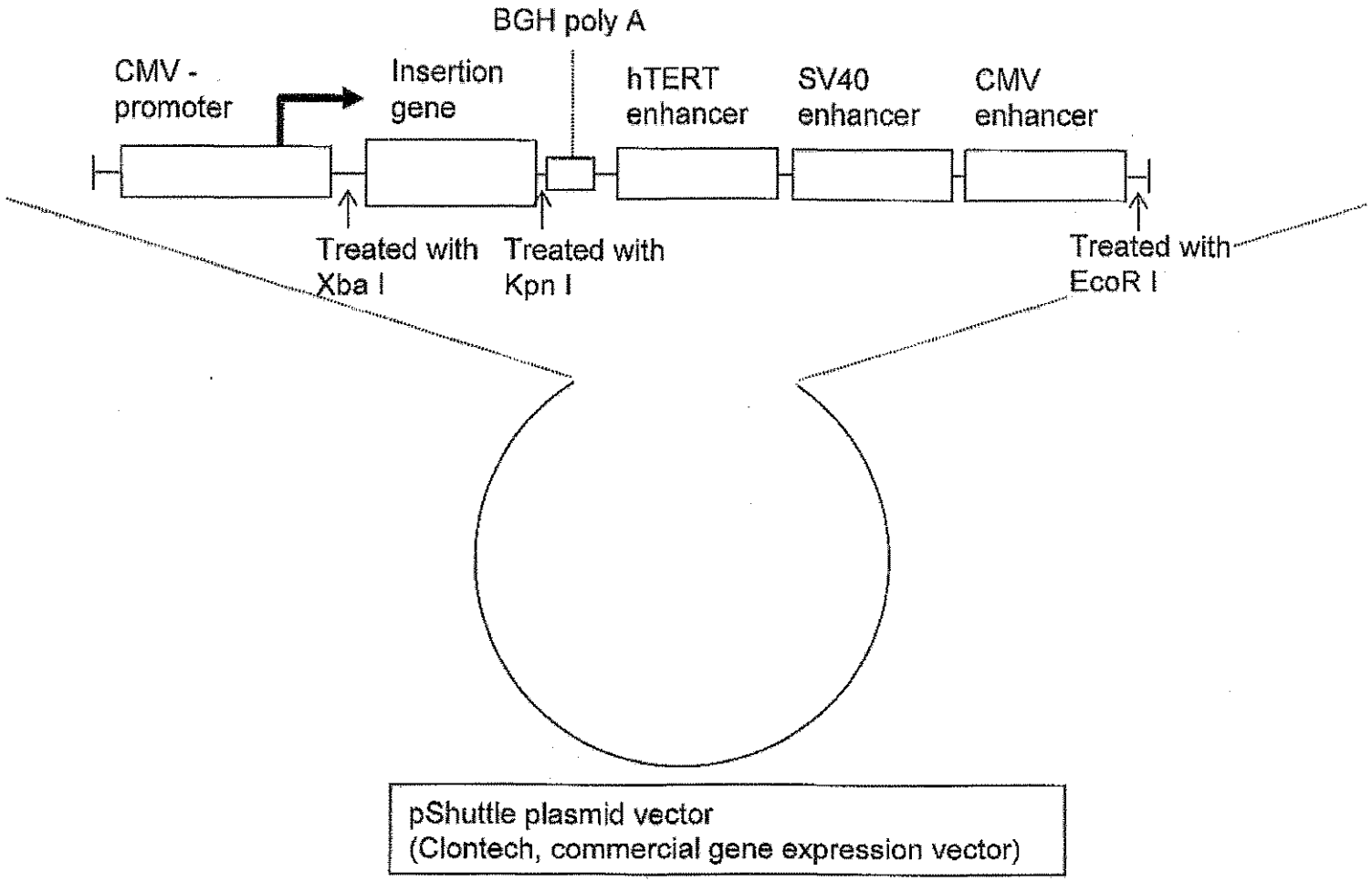


Fig. 50

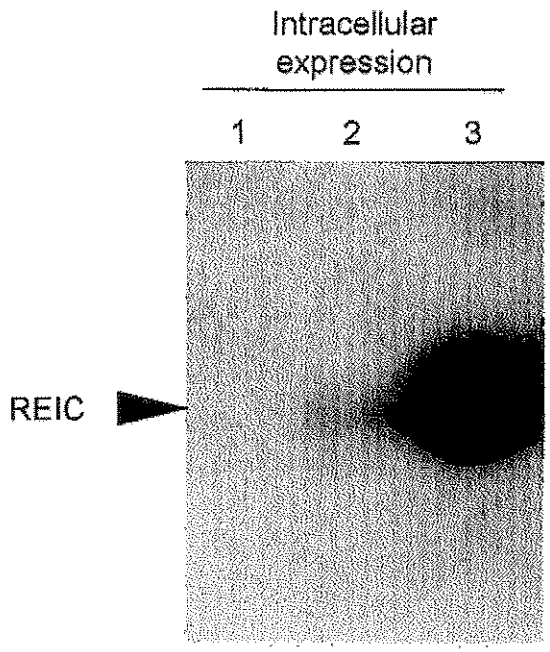
pshuttle- REIC-TSC

(1) (2)

XbaI-REIC-KpnI-3xenh-EcoRI

T/CTAGAGCacc**atg**cagcggcttggggccaccctgctgtgcctgctgctggcggcggcggt (1)
ccccacggcccccgcgcccgctccgacggcgacctcggctccagtcaagcccggcccggctc
tcagctacccgcaggaggaggccaccctcaatgagatgttccgcgaggttgaggaactgatg
gaggacacgcagcacaaattgcgcagcgcggtggaagagatggaggcagaagaagctgctgc
taaagcatcatcagaagtgaacctggcaaacttacctcccagctatcacaatgagaccaaca
cagacacgaaggttggaaataataccatccatgtgcaccgagaaattcacaagataaccaac
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cagaaggagccacgagtgcatcatcgacgaggactgtgggcccagcatgtactgccagtttg
ccagcttccagtacacctgccagccatgccggggccagaggatgctctgcacccgggacagt
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ccttgtgccagtggcctcctctgccagccccacagccacagcctggtgtatgtgtgcaagc
cgaccttcgtggggagccgtgaccaagatggggagatcctgctgcccagagaggtccccgat
gagtatgaagttggcagcttcatggaggaggtgcgccaggagctggaggacctggagaggag
cctgactgaagagatggcgctgggggagcctgcggctgccgccgctgcactgctgggagggg
aagagatt**tag**GG**GGTAC/C**CCG**GC**tagatgactaacGTTTAAACCCGCTGATCAGCCTCGA
CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTG
GAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAG
TAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAG
ACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG**cggagtactgtcctccg**cttccc (2)
acgtggcggagggactggggacccgggcacccgtcctgcccccttcaccttccagctccgcct
cctccgcgcggaccccgccccgtcccgacccctcccgggtccccggcccagcccccctccggg
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AAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAA
CCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAAT
TAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTC
CGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCT
CTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCAAGGCTTTTGCAAA
AAGCTCcgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcc
cattgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgt
caatgggtggagtatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgcc
aagtacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtaca
tgaccttatgggactttcctacttggcagtacatctacgtattagtcatcgctattaccatg
gtgatgcggttttggcagtacatcaatgggcgtggatagcggtttgactcacggggatttcc
aagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggactttc
caaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgTTG**CCGG/AAT**
TC

Fig. 51



1, Untreated cell sample
2, pShuttle(Commercial expression plasmid)
3, Plasmid constructed by addition of 3 enhancers (existing downstream of the expression gene of expression plasmid construct No. 14) to a site downstream (downstream of BGH poly A) of the expression gene of a pShuttle plasmid.

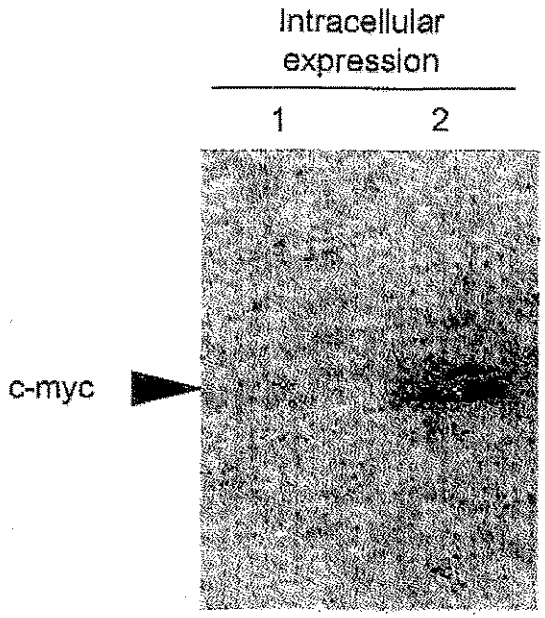
Fig. 52

atgttcatgccttcttcttttttcctacagctcctgggcaacgtgctggttattgtgctgt
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aatttctatcaccagcaacagcagagcgagctgcagccgcccgcgcccagtgaggatatc
tggaagaaattcgagctgcttcccaccccgcccctgtccccgagccgccgctccgggctc
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acggaggaaaacgacaagaggcggacacacaacgtcttggaacgtcagaggaggaacgag
ctgaagcgcagcttttttgccctgcgtgaccagatccctgaattggaaaacaacgaaaag
gcccccaaggtagtgatcctcaaaaaagccaccgcctacatcctgtccattcaagcagac
gagcacaagctcacctctgaaaaggacttattgaggaaacgacgagaacagttgaaacac
aaactcgaacagcttcgaaactctggtgcataa

Fig. 53

GTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCC
CTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATG
AGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAG
GACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTAT
GGCGGAGTACTGTCCTCCGCTTCCCACGTGGCGGAGGGACTGGTCCTCCGCTTCCCACGTGG
CGGAGGGACTGGGGACCCGGGCACCCGTCCTGCCCCTTCACCTTCCAGCTCCGCCTCCTCCG
CGCGGACCCCGCCCCGTCCCGACCCCTCCCGGGTCCCCGGCCCAGCCCCCTCCGGGCCCTCC
CAGCCCCTCCCCTTCCTTTCCGCGGCCCCGCCCTCTCCTCGCGGCGCGAGTTTTGGAAAGTC
CCCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAACCAGGT
GTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCA
GCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCA
TTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCT
CTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCAAGGCTTTTGCAAAAAGCTC
CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGA
CGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGG
GTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTAC
GCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCT
TATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATG
CGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCT
CCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAAT
GTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTG

Fig. 54



1, Gene expression plasmid such as pShuttle
2, Plasmid constructed by insertion of BGH poly A and 3 enhancers (existing downstream of the expression gene of expression plasmid construct No. 14) into a site downstream of the expression gene of the plasmid 1 above.

Fig. 55

Construct No.18

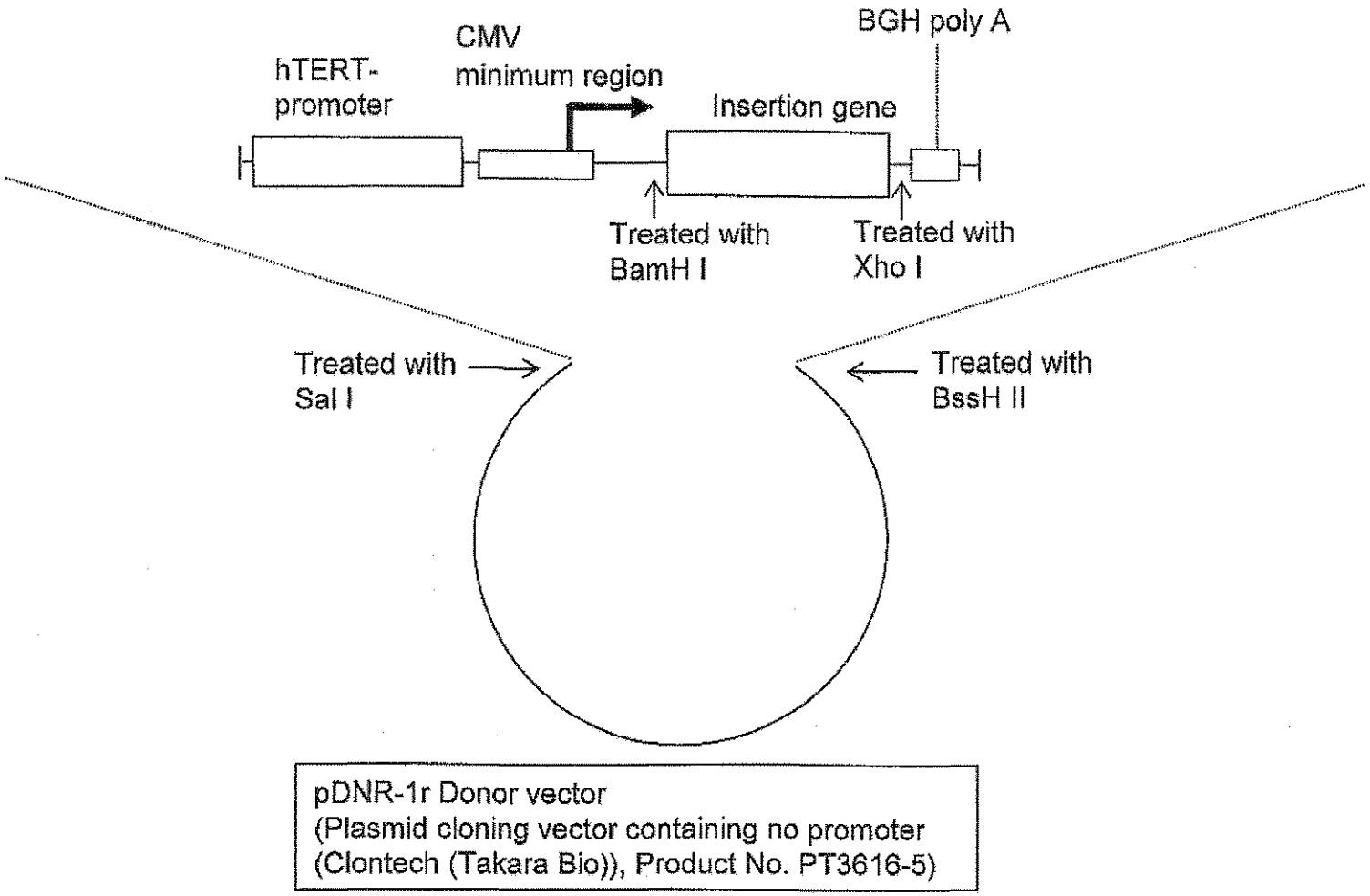


Fig. 56

Construct No.19

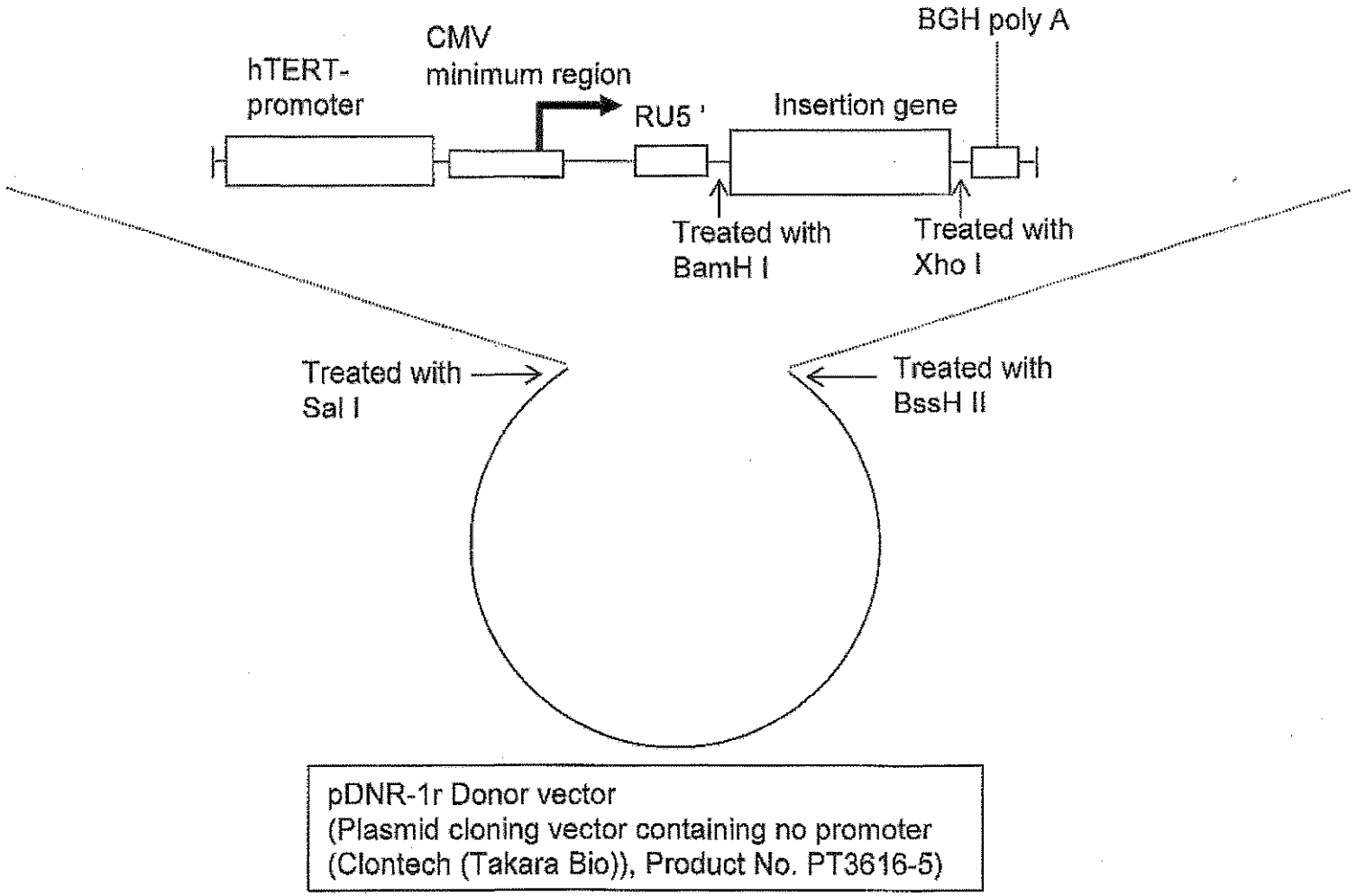


Fig. 57

Construct No.20

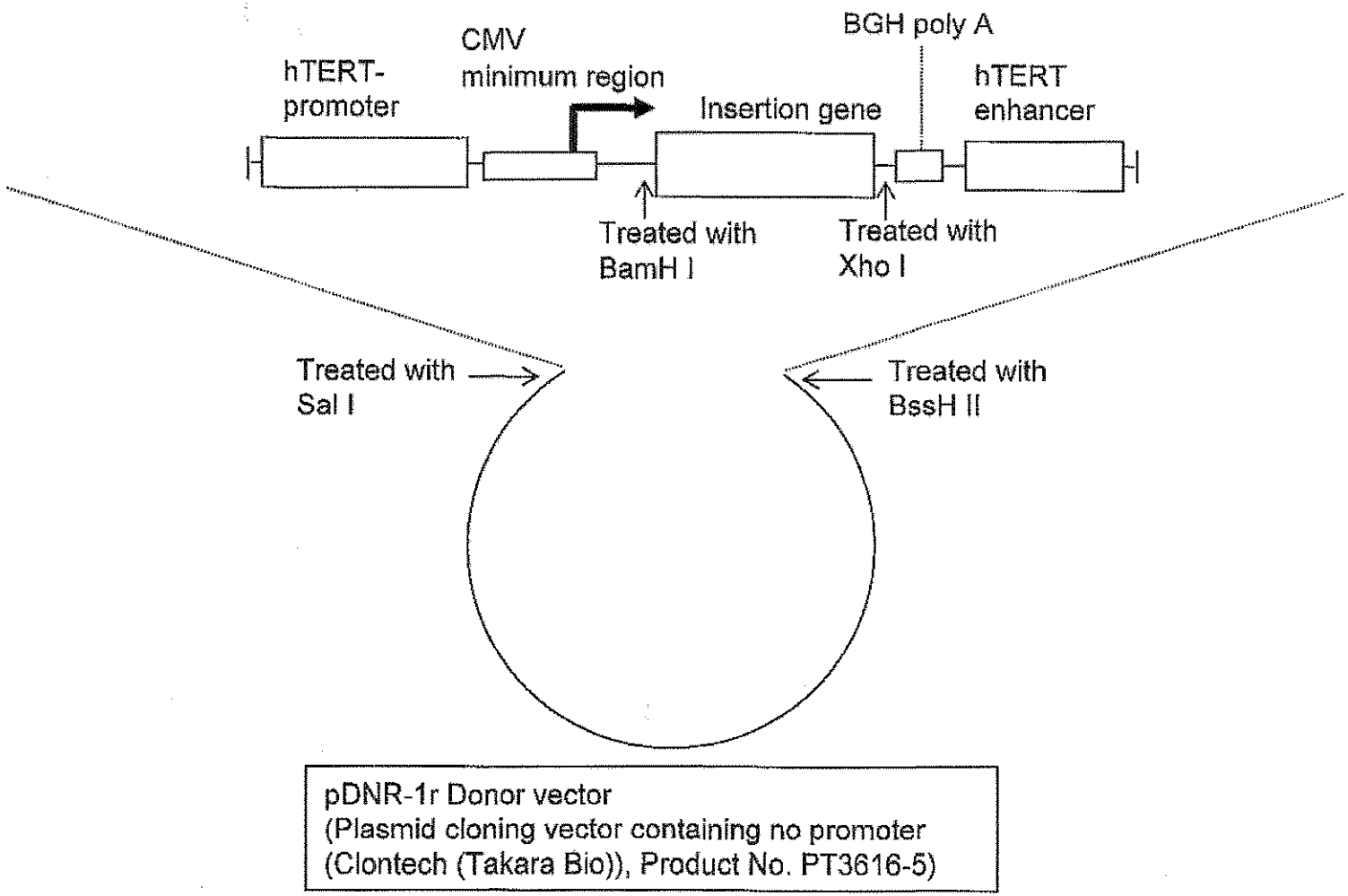


Fig. 58

Construct No.21

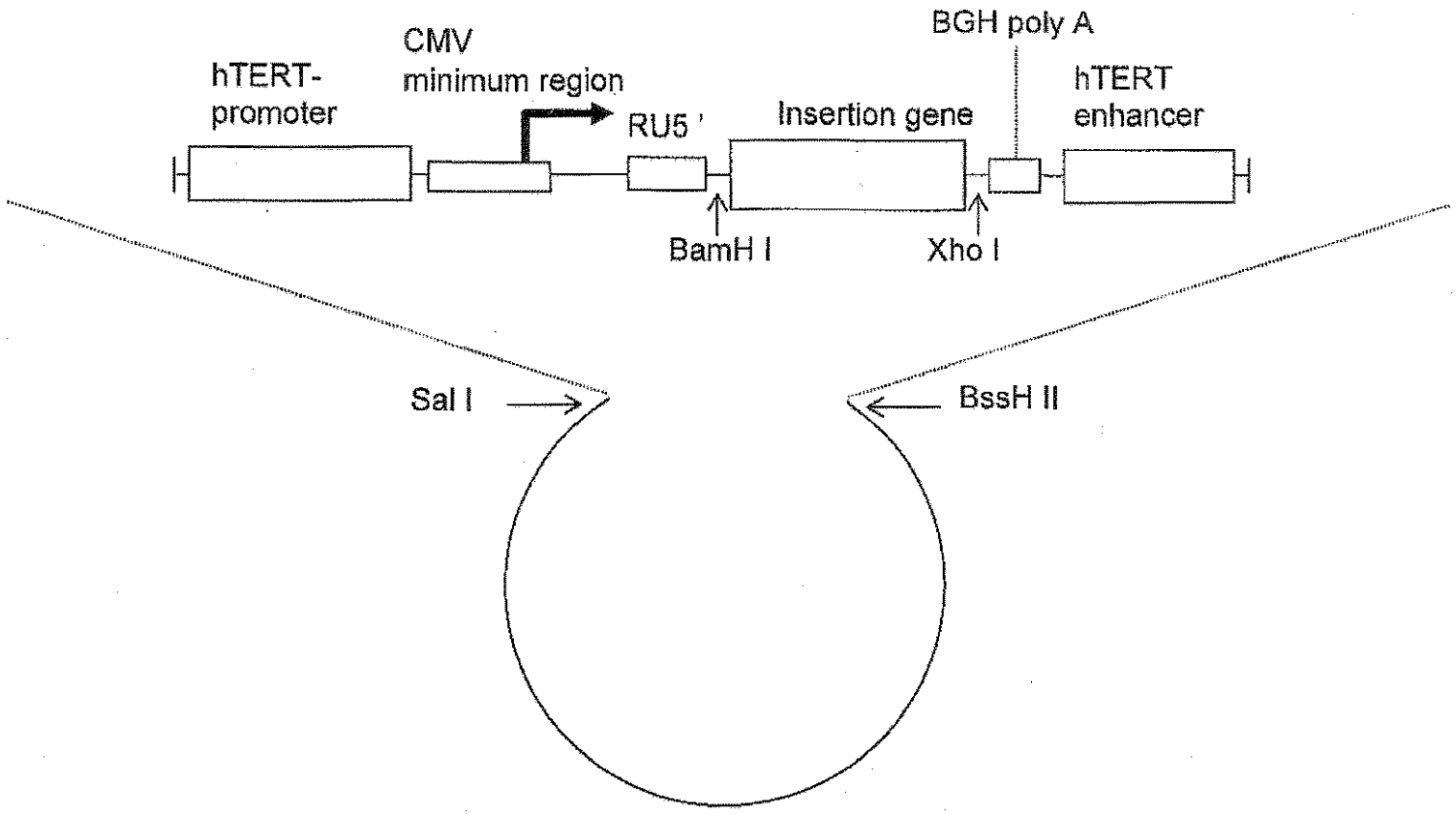


Fig. 59-1

#21-GFP
pDNR-1r (Sal-BssHII)

(1) (2)
Sal1-EcoR1-**hTERT core promoter**-minimal CMV promoter-
(3) (4) (5)
HindIII-RU5'-HindIII-BamH1-**GFP**-Xho1-Xba1-**BGHpA**-BstXI-
(6)
hTERT core promoter -BssHII

GTCGACGTCGGCCATA CCGGAATTCCGG_

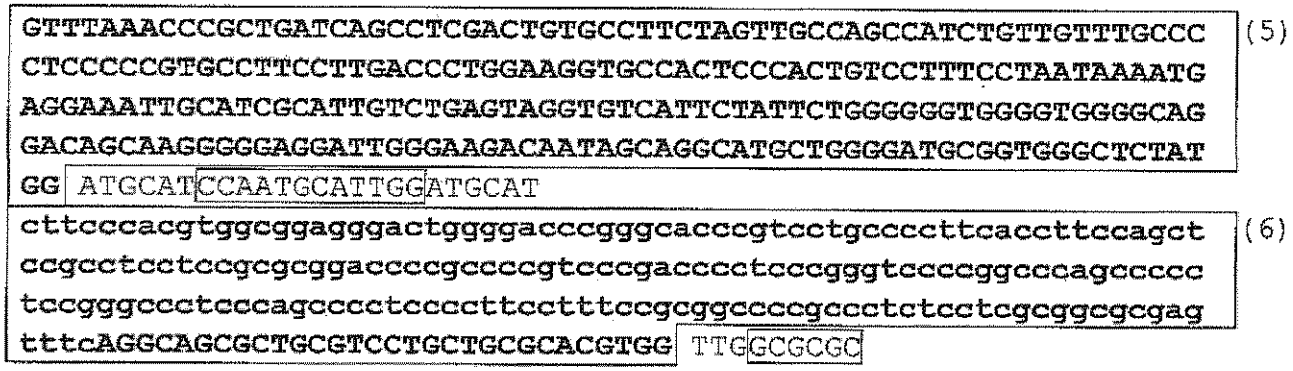
cttcccacgtggcggagggactggggacccgggcacccgtcctgccccttcaccttccagct (1)
ccgcctcctccgcgcggaccccgccccgtcccgacccctcccgggtccccggcccagccccc
tccgggccctcccagccccctcccctttcctttccgcggccccgccctctcctcgcggcgcgag
tttcAGGCAGCGCTGCGTCCTGCTGCGCACGTGG

GGTAGGCGTGTACGGTGGGAGGCCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGC (2)
CTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCC
GCGGCCCCGCATTCGAGCTCGGTACCCGG CCCAAGCTTGGG

agcttcgaggggctcgcatctctccttcacgcgcccgccgccctacctgaggccgccatcca (3)
cgccggttgagtcgcgttctgccgcctcccgcctgtggtgcctcctgaactgcgtccgccgt
ctaggtaagtttaaagctcaggtcgagaccgggcctttgtccggcgctcccttggagcctac
ctagactcagccggctctccacgctttgcctgaccctgcttgctcaactctacgtctttgtt
tcgttttctgttctgcgccgttacagatccaagccacc CCCAAGCTTGGG CGGGATCCCG

acc **atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc** (4)
ctg gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc
ggc gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc
atc tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc
acc ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg
aag cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag
gag cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc
gag gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag
ggc atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag
tac aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag
aac ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc
agc gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac
ggc ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc
ctg agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag
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tga CCGCTCGAGCGG GCTCTAGAG

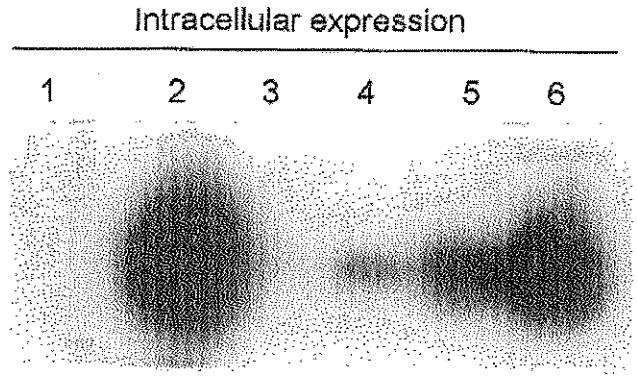
Fig. 59-2


GTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCC
CTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATG
AGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAG
GACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTAT
GG ATGCATCCAATGCATTGGATGCAT
cttcccacgtggcggagggactggggacccgggcacccgtcctgcccccttcaccttccagct
ccgcctcctccgcgcggaccccgccccgtcccgaccccctcccgggtccccggcccagccccc
tccgggccctcccagccccctcccctttcctttccgcggcccccgccctctcctcgcggcgcgag
tttcAGGCAGCGCTGCGTCCTGCTGCGCACGTGG TTGGCGCGC

(5)

(6)

Fig. 60



1, Untreated cell sample
2, Expression plasmid construct No. 2
3, pTERT-CMVmin(Construct No.18)
4, pTERT-CMVmin-RU5'(Construct No.19)
5, pTERT-CMVmin / pTERT(Construct No.20)
6, pTERT-CMVmin-RU5'/ pTERT(Construct No.21)

Fig. 61

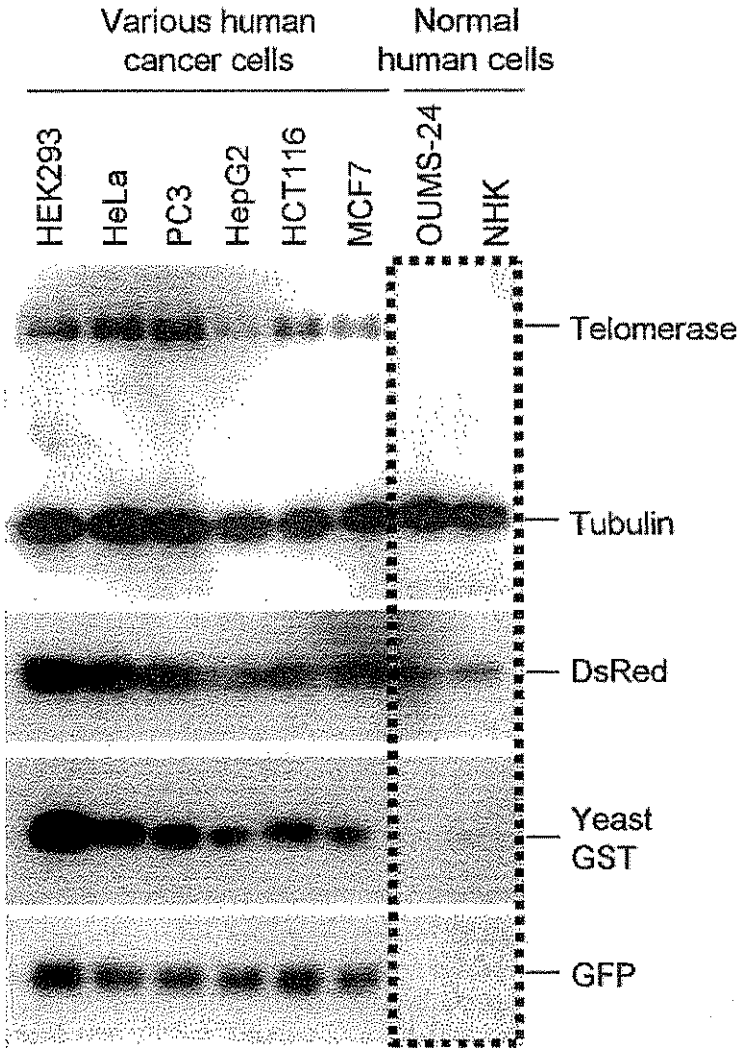
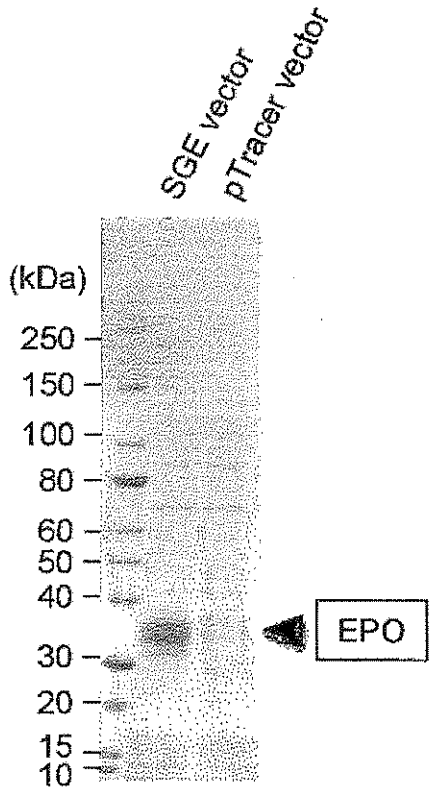
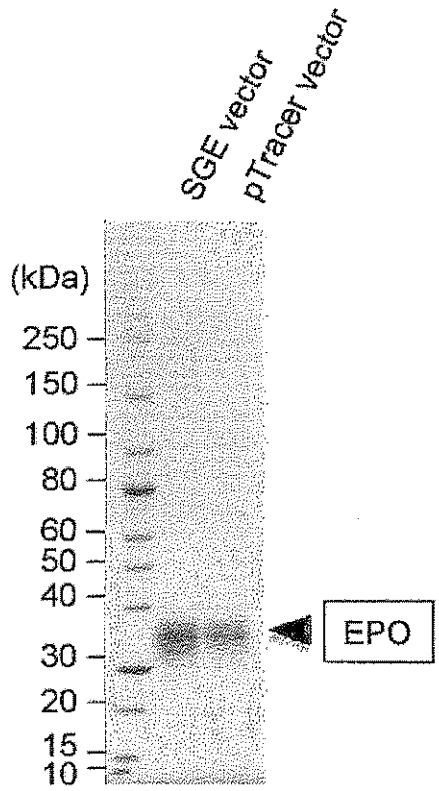

Various human cancer cells
Normal human cells
HEK293
HeLa
PC3
HepG2
HCT116
MCF7
OUMS-24
NHK
Telomerase
Tubulin
DsRed
Yeast GST
GFP

Fig. 62



CBB staining

Fig. 63



CBB staining

Fig. 64

A

Amount of protein purified by histidine affinity column chromatography from 25 mL of culture supernatant

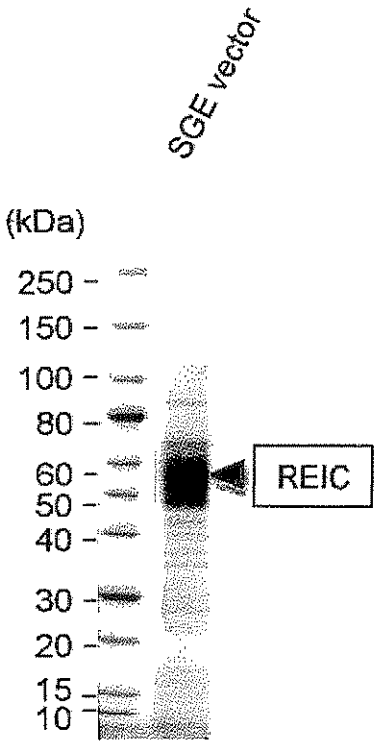
SGE vector	3.83mg
pTracervector	0.49mg

B

Amount of protein collected in terms of 1 L of culture supernatant

SGE vector	153.2mg
pTracer vector	19.6mg

Fig. 65



CBB staining

Fig. 66

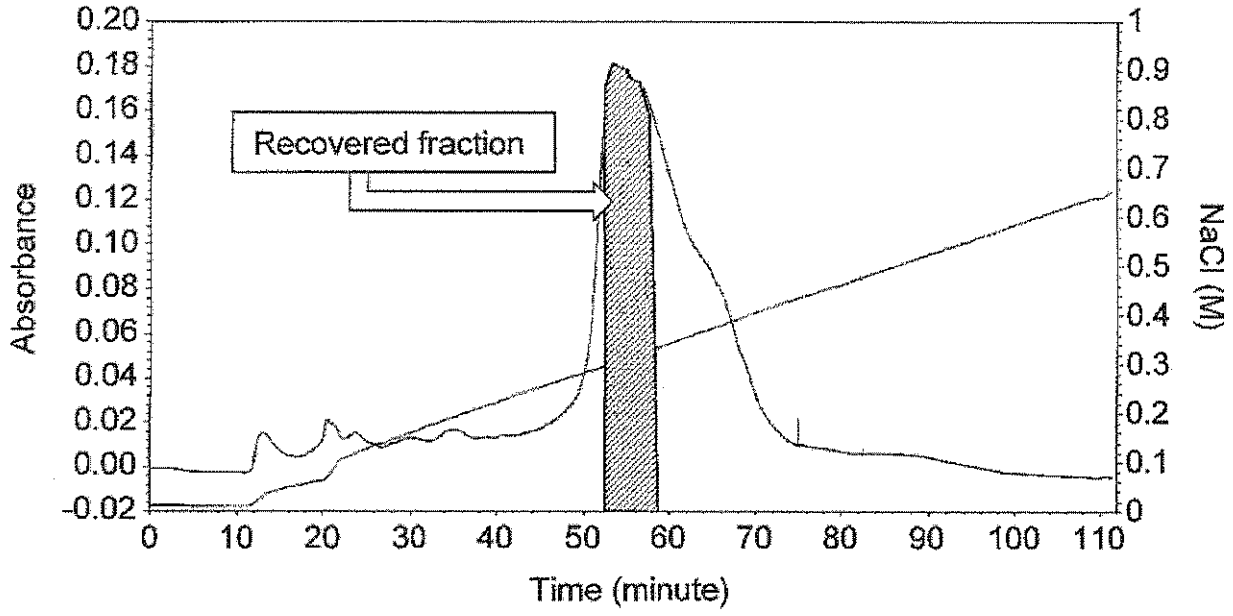

Recovered fraction
Absorbance
NaCl (M)
Time (minute)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
80
90
100
110

Fig. 67

Amount of protein purified from culture supernatant obtained after transfection with REIC expression SGE vector

Amount of protein purified from 520 mL of culture supernatant	51mg
Amount of protein collected in terms of 1 L of culture supernatant	98mg