図 1

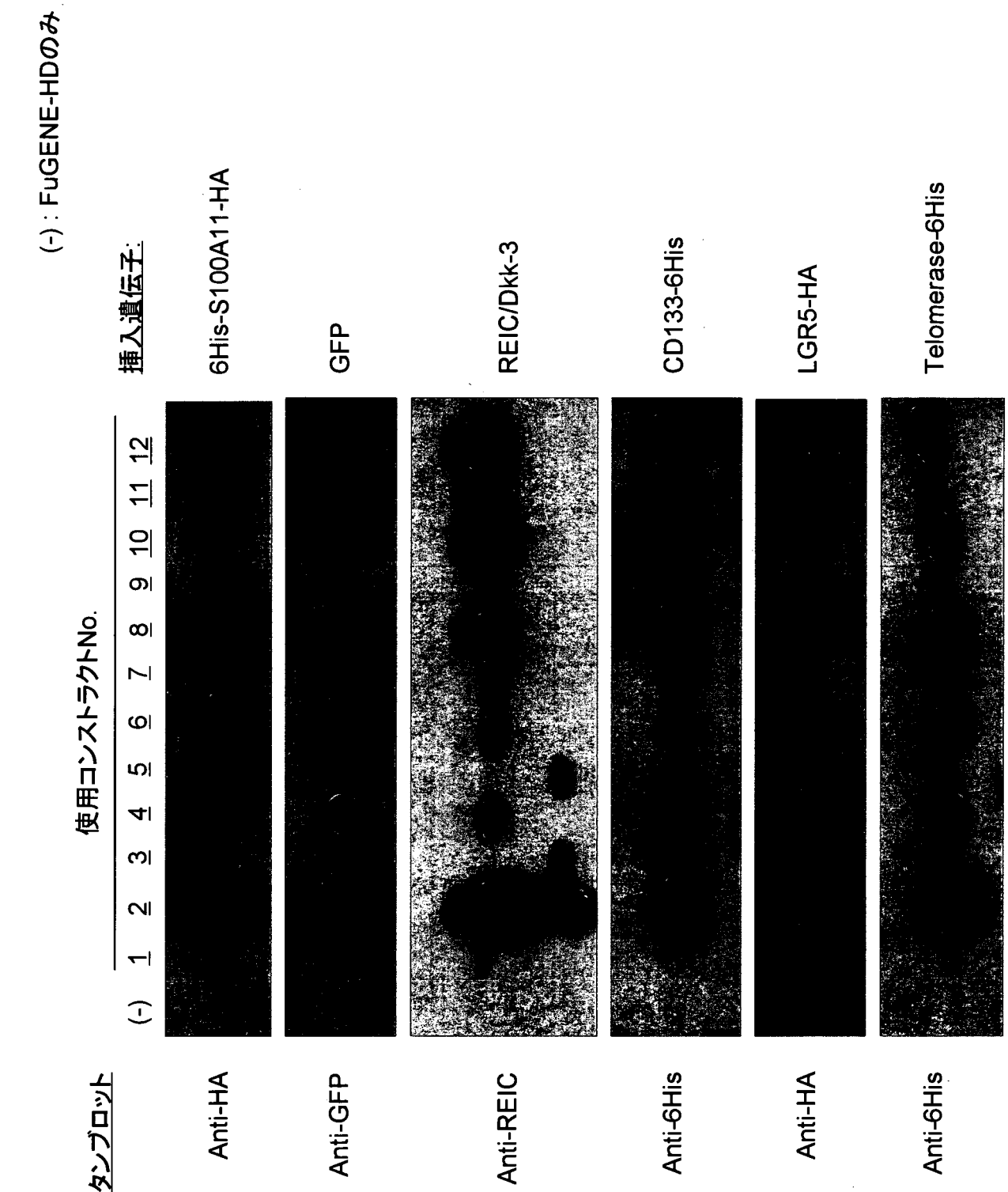

(-) : FuGENE-HDのみ
ウエスタンブロット分析:
使用コンストラクトNo.
(-) 1 2 3 4 5 6 7 8 9 10 11 12
挿入遺伝子:
Anti-HA
6His-S100A11-HA
Anti-GFP
GFP
Anti-REIC
REIC/Dkk-3
Anti-6His
CD133-6His
Anti-HA
LGR5-HA
Anti-6His
Telomerase-6His

図2

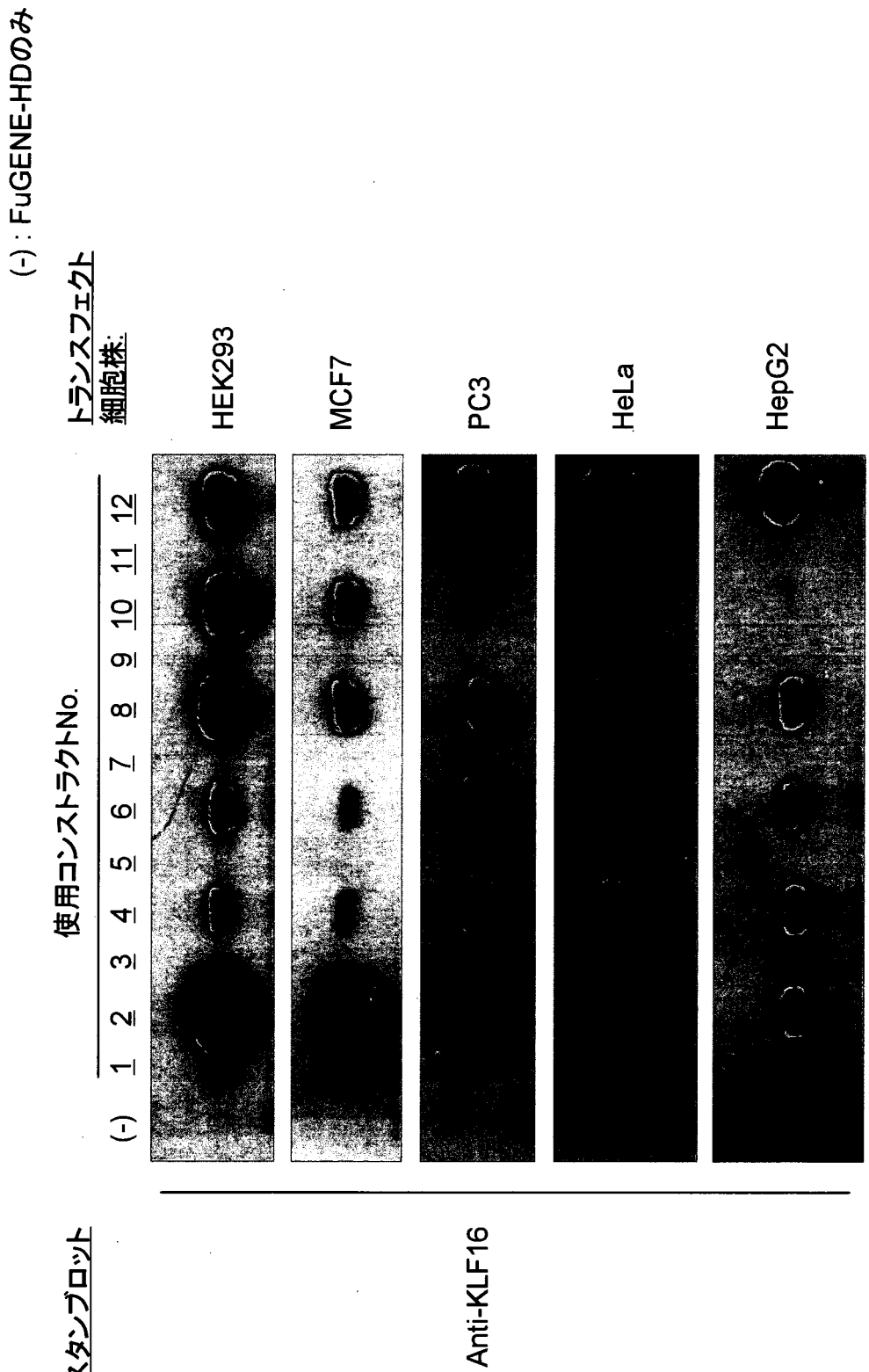

(-) : FuGENE-HDのみ
トランスフェクト
細胞株:
HEK293
MCF7
PC3
HeLa
HepG2
使用コンストラクトNo.
(-) 1 2 3 4 5 6 7 8 9 10 11 12
ウエスタンブロット
分析:
Anti-KLF16

図3

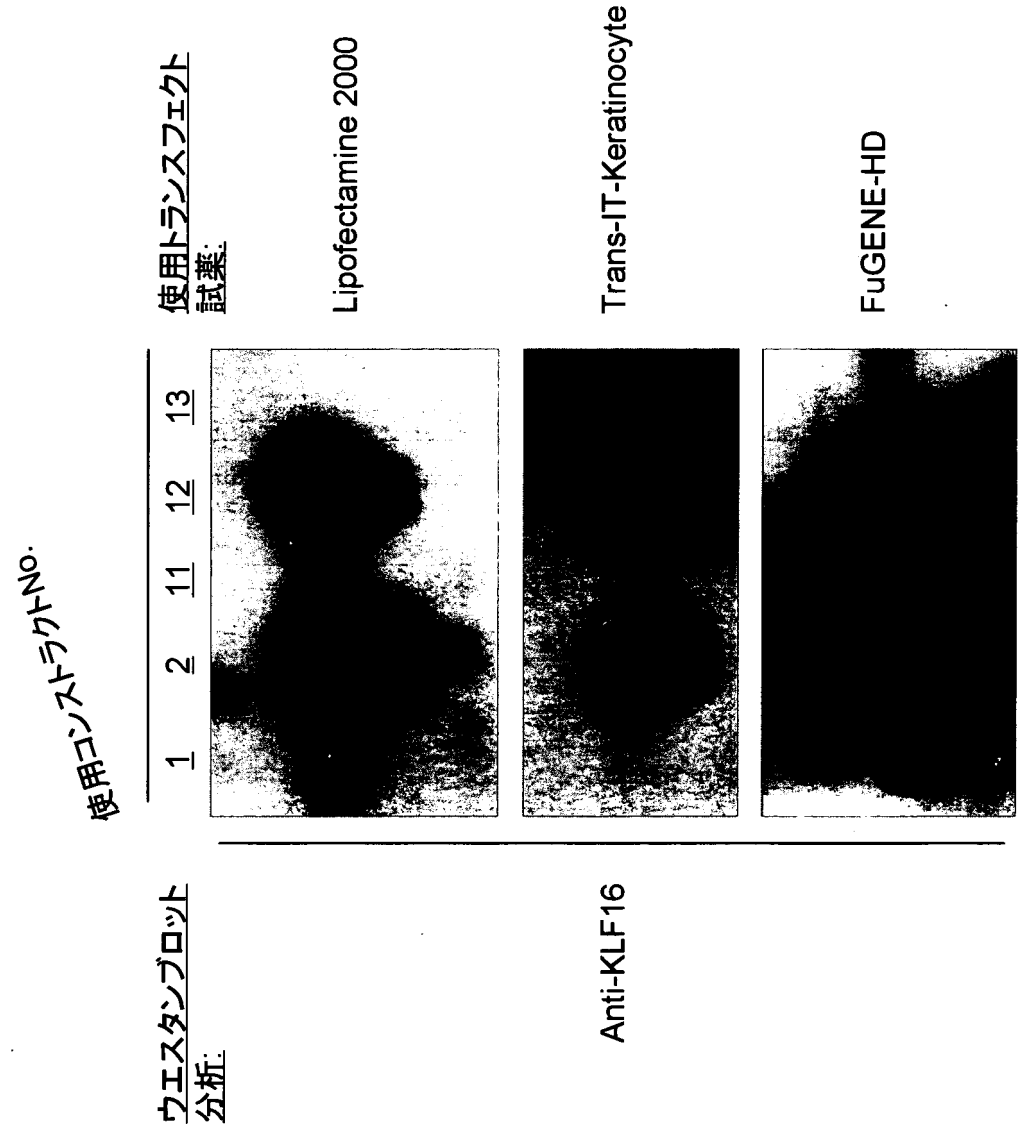
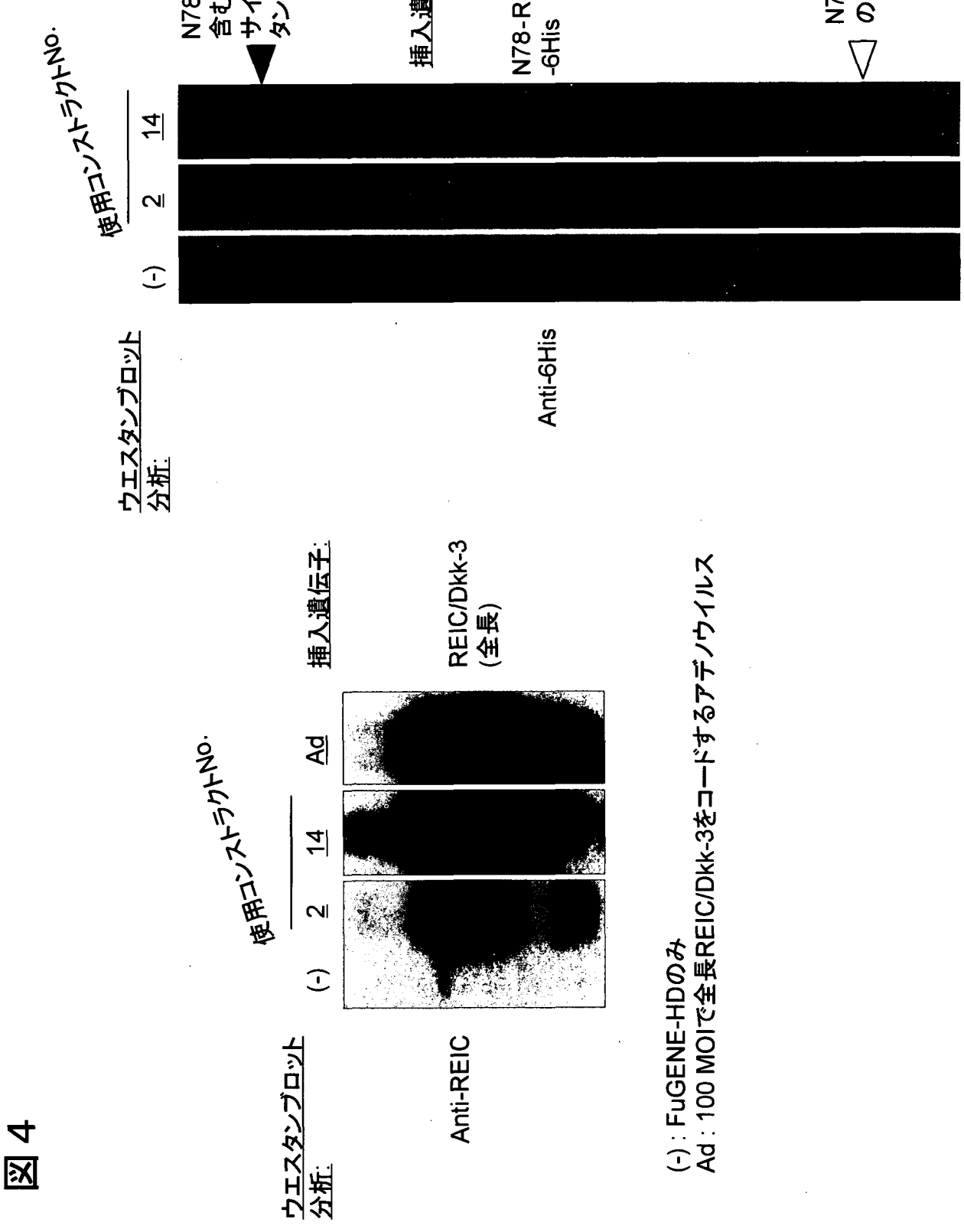

使用コンストラクトNo.
1
2
11
12
13
ウエスタンブロット分析:
Anti-KLF16
使用トランスフェクト試薬:
Lipofectamine 2000
Trans-IT-Keratinocyte
FuGENE-HD

図 4



(-) : FuGENE-HDのみ
Ad : 100 MOIで全長REIC/Dkk-3をコードするアデノウイルス

図５－１

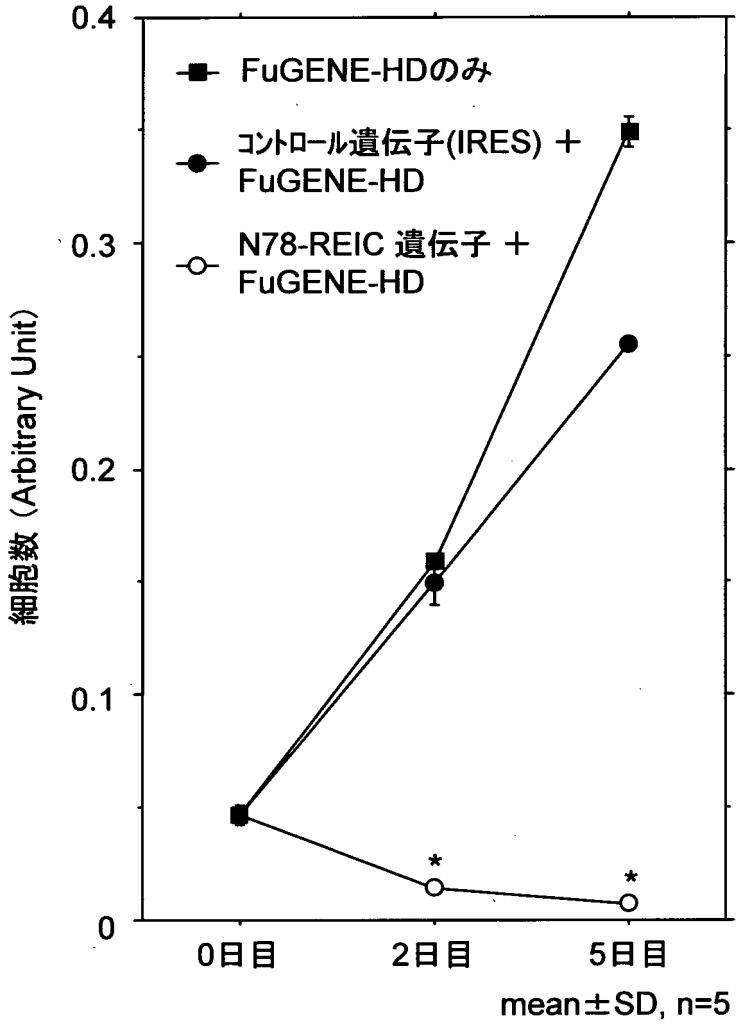
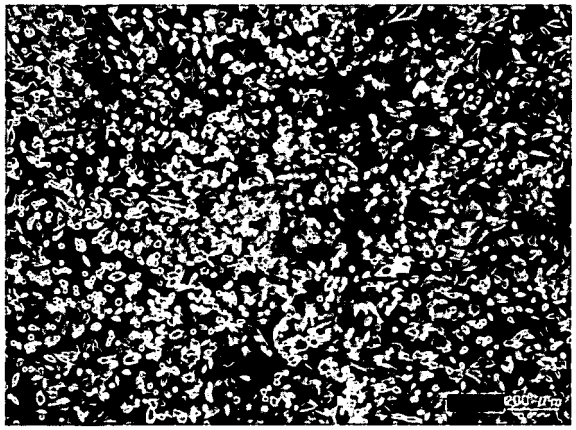

0.4
0.3
0.2
0.1
0
細胞数 (Arbitrary Unit)
FuGENE-HDのみ
コントロール遺伝子(IRES) ＋
FuGENE-HD
N78-REIC 遺伝子 ＋
FuGENE-HD
*
*
0日目
2日目
5日目
mean±SD, n=5

図５－２

A

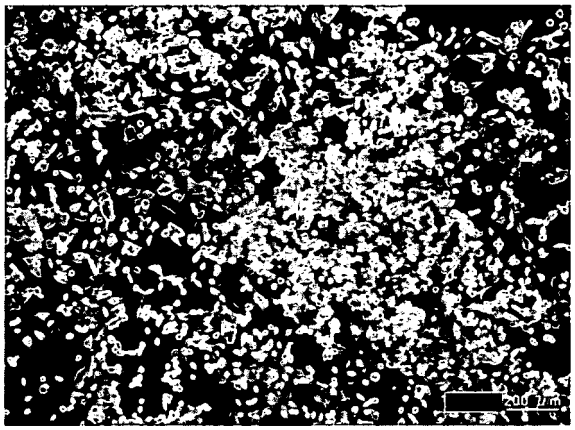
FuGENE-HDのみ
（5日目）



Bar = 200 μm

B

コントロール遺伝子 (IRES)
＋ FuGENE-HD
（5日目）



C

N78-REIC遺伝子
＋ FuGENE-HD
（5日目）

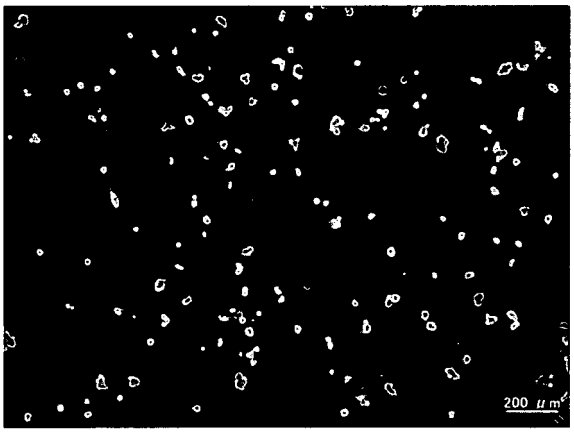


図6

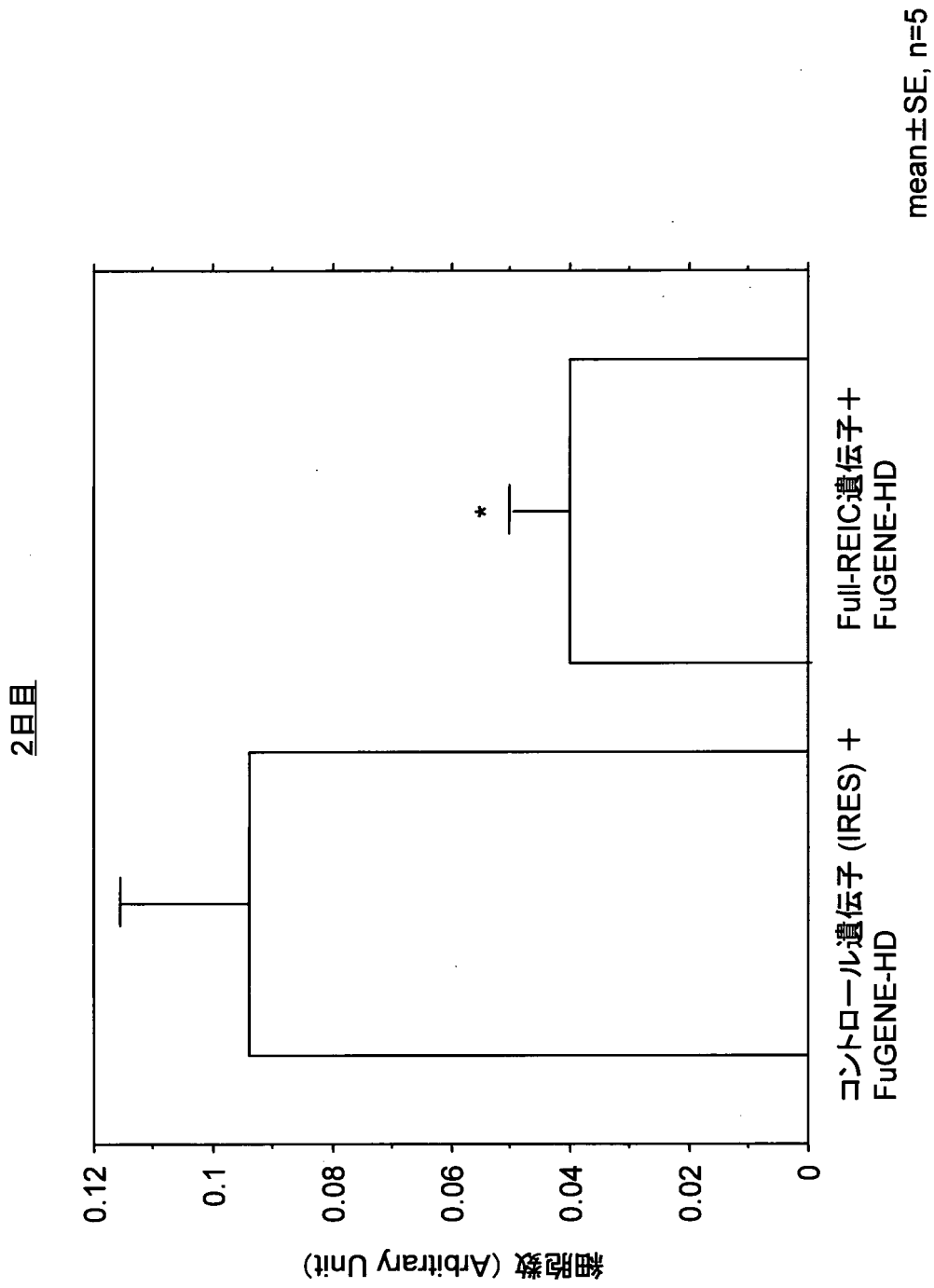

2日目
細胞数 (Arbitrary Unit)
0.12
0.1
0.08
0.06
0.04
0.02
0
*
コントロール遺伝子 (IRES) + FuGENE-HD
Full-REIC遺伝子＋ FuGENE-HD
mean±SE, n=5

図 7

コンストラクト No.1

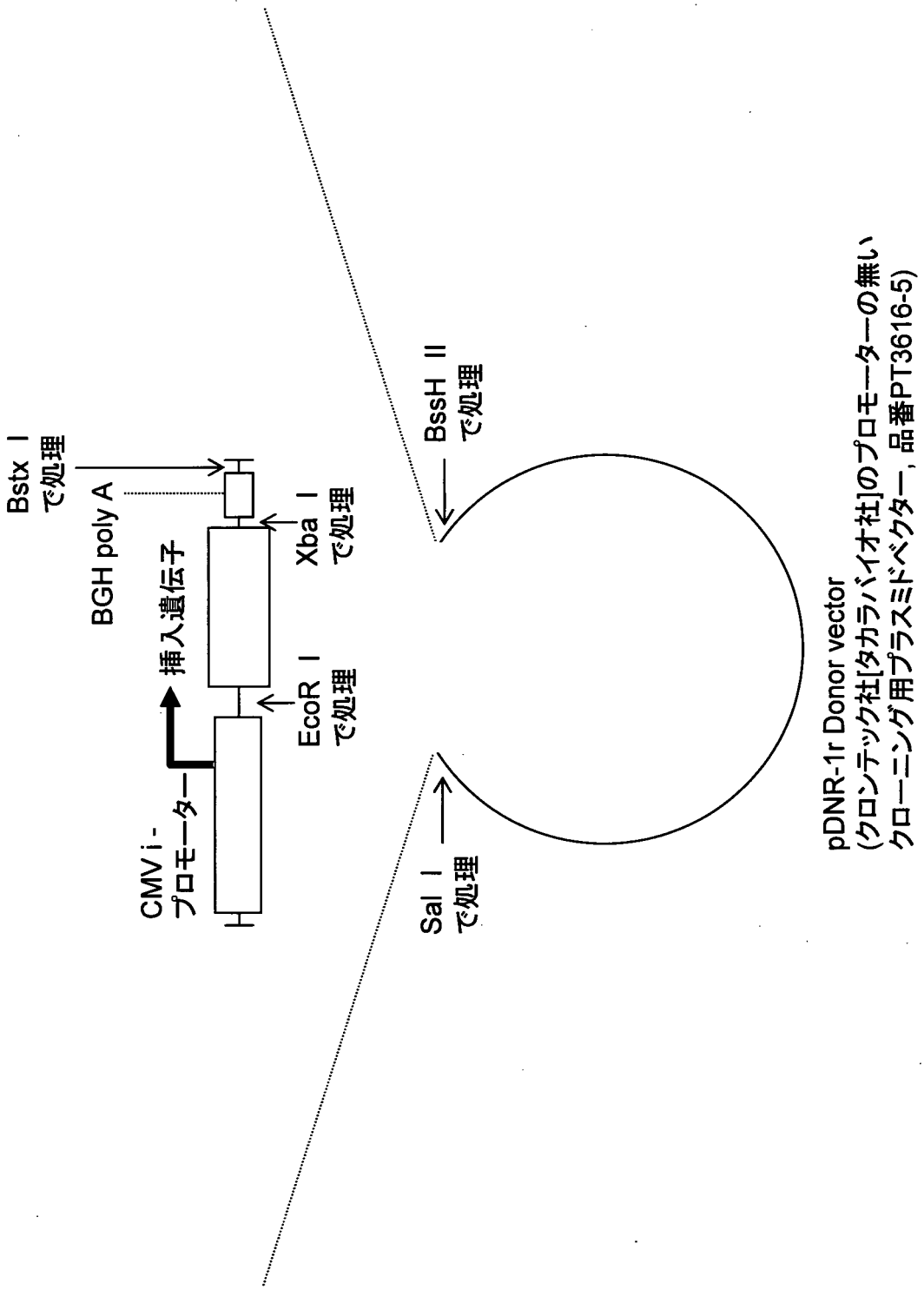


図 8
コンストラクト No.2

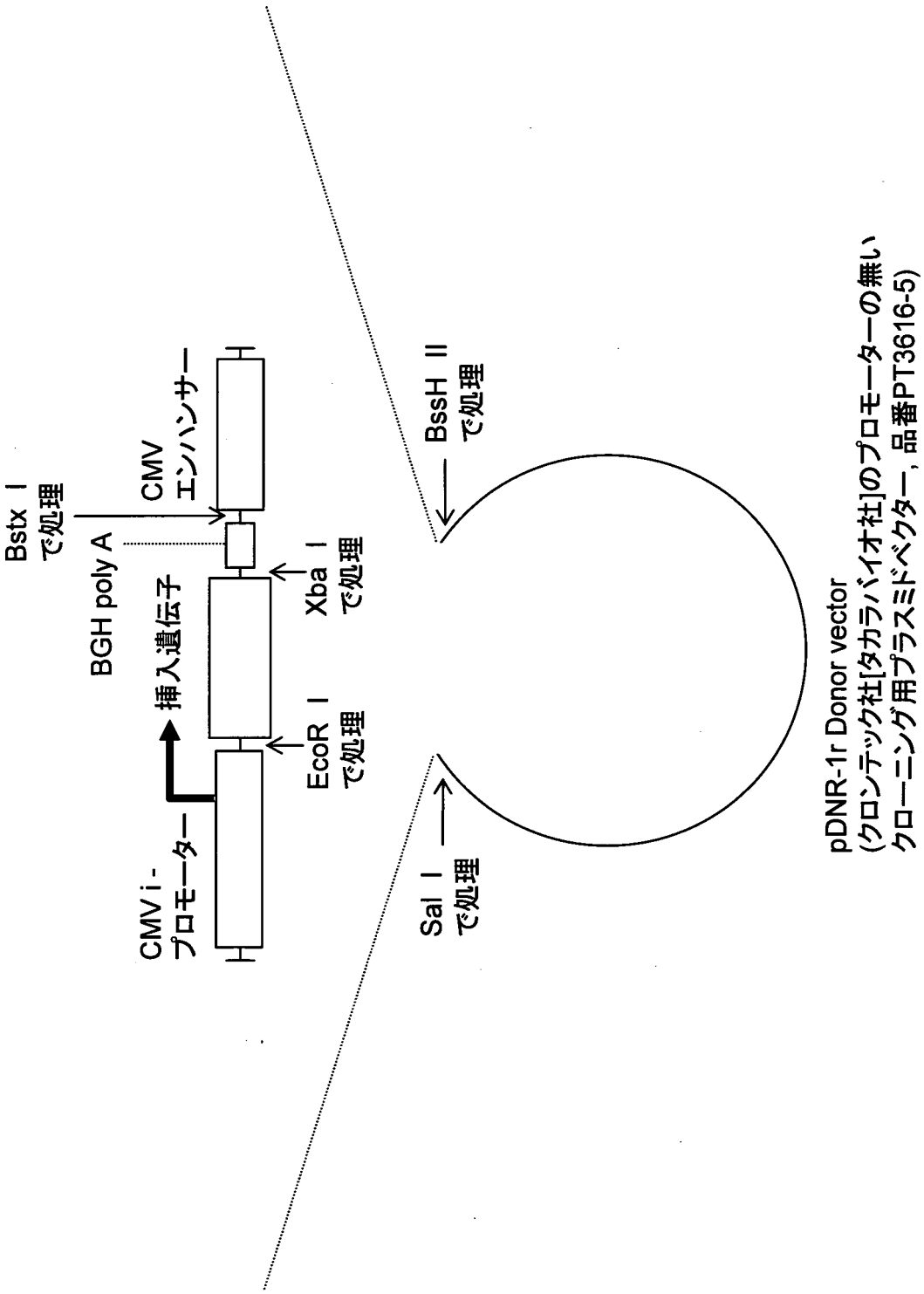


図 9

コンストラクト No.3

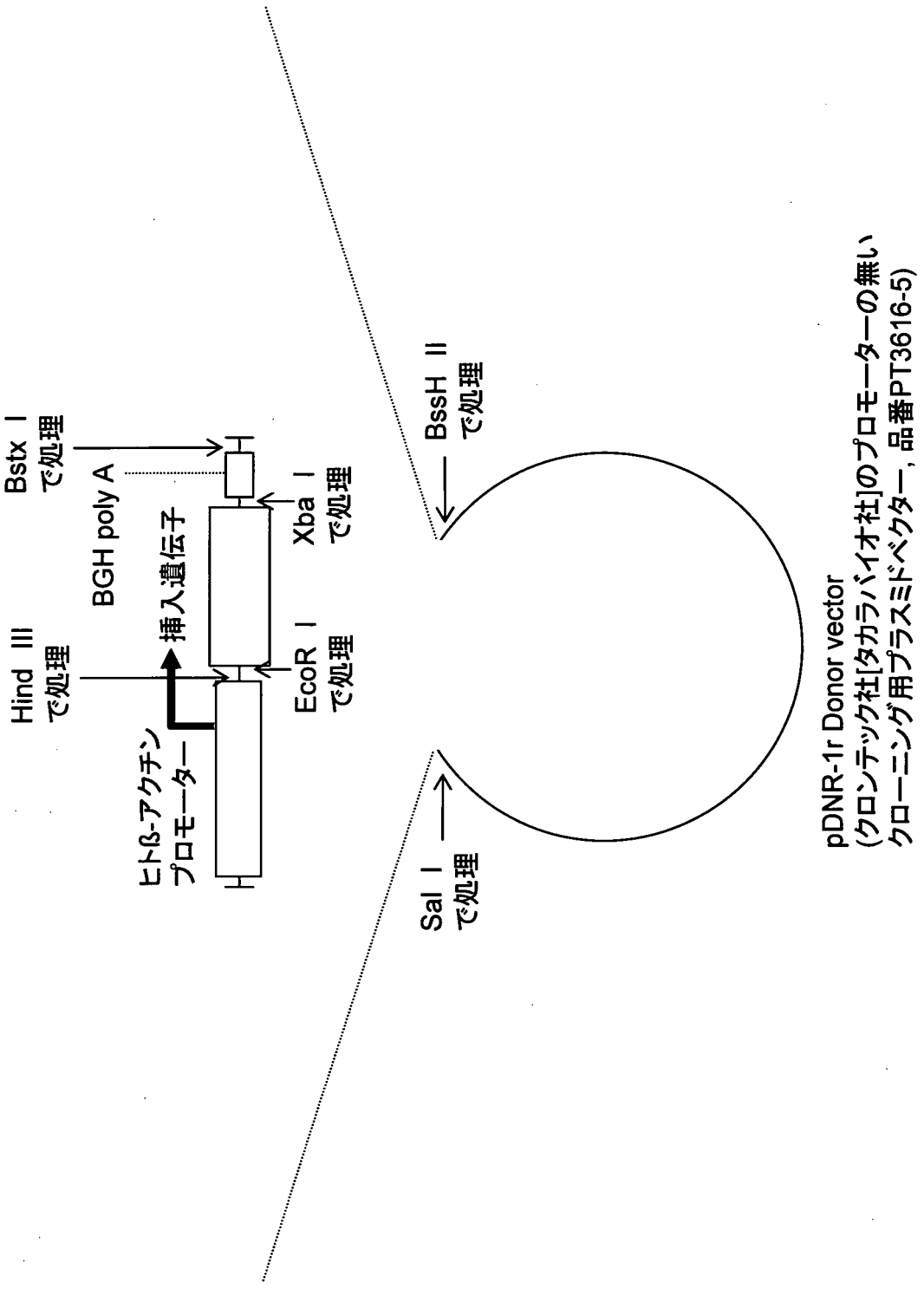


図１０
コンストラクト No.4

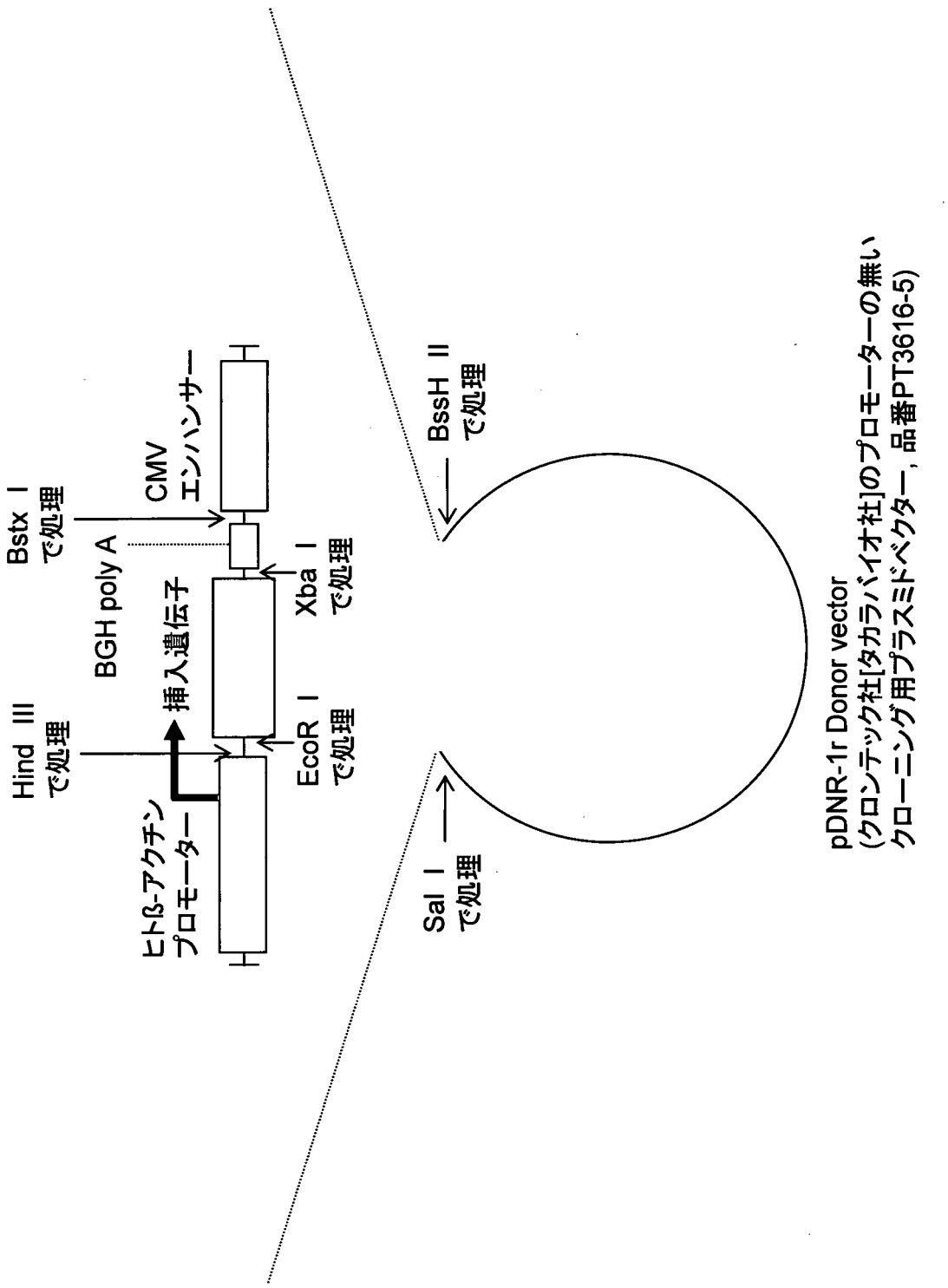


図１１
コンストラクト No.5

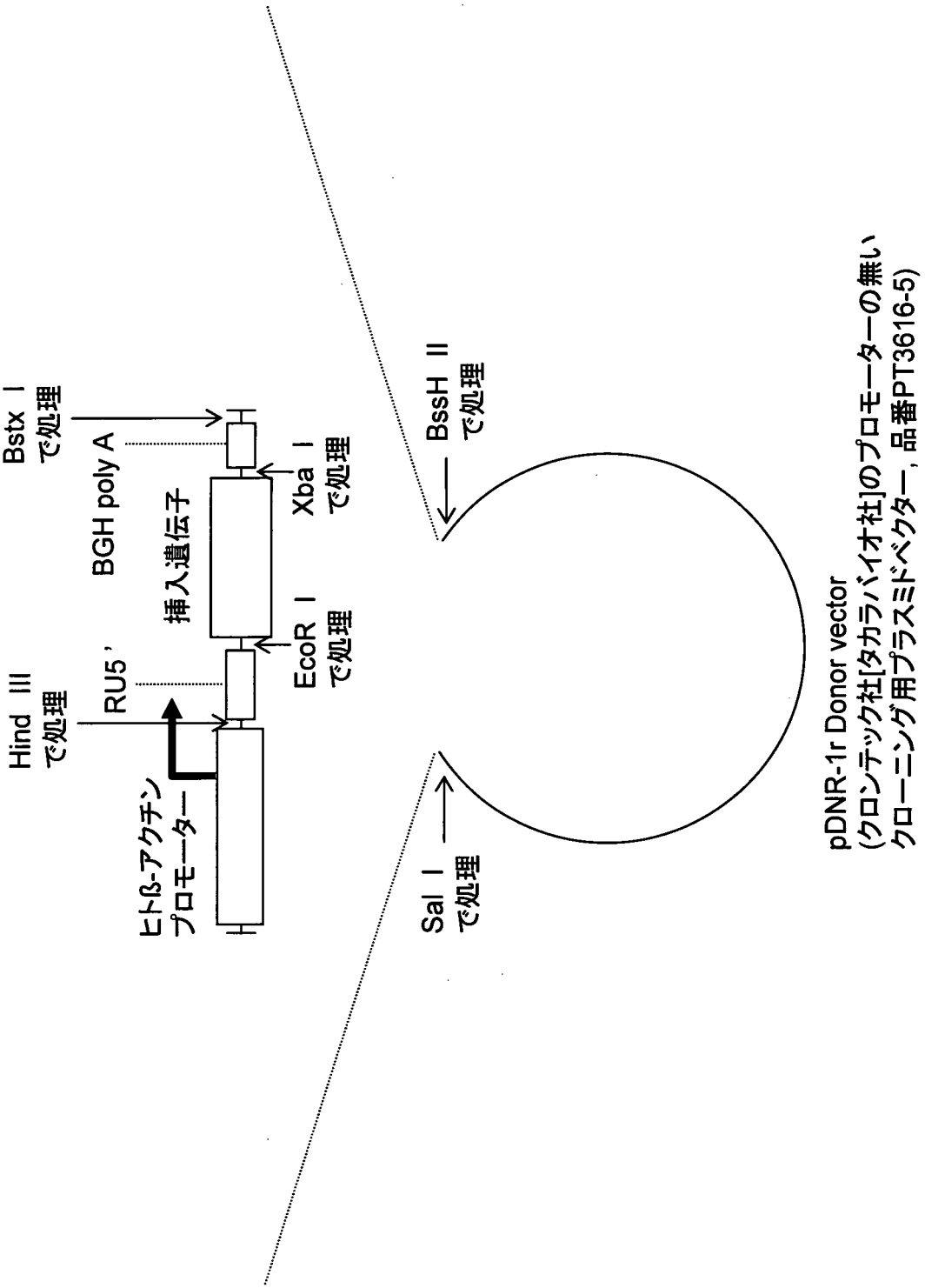


図１２
コンストラクト No.6

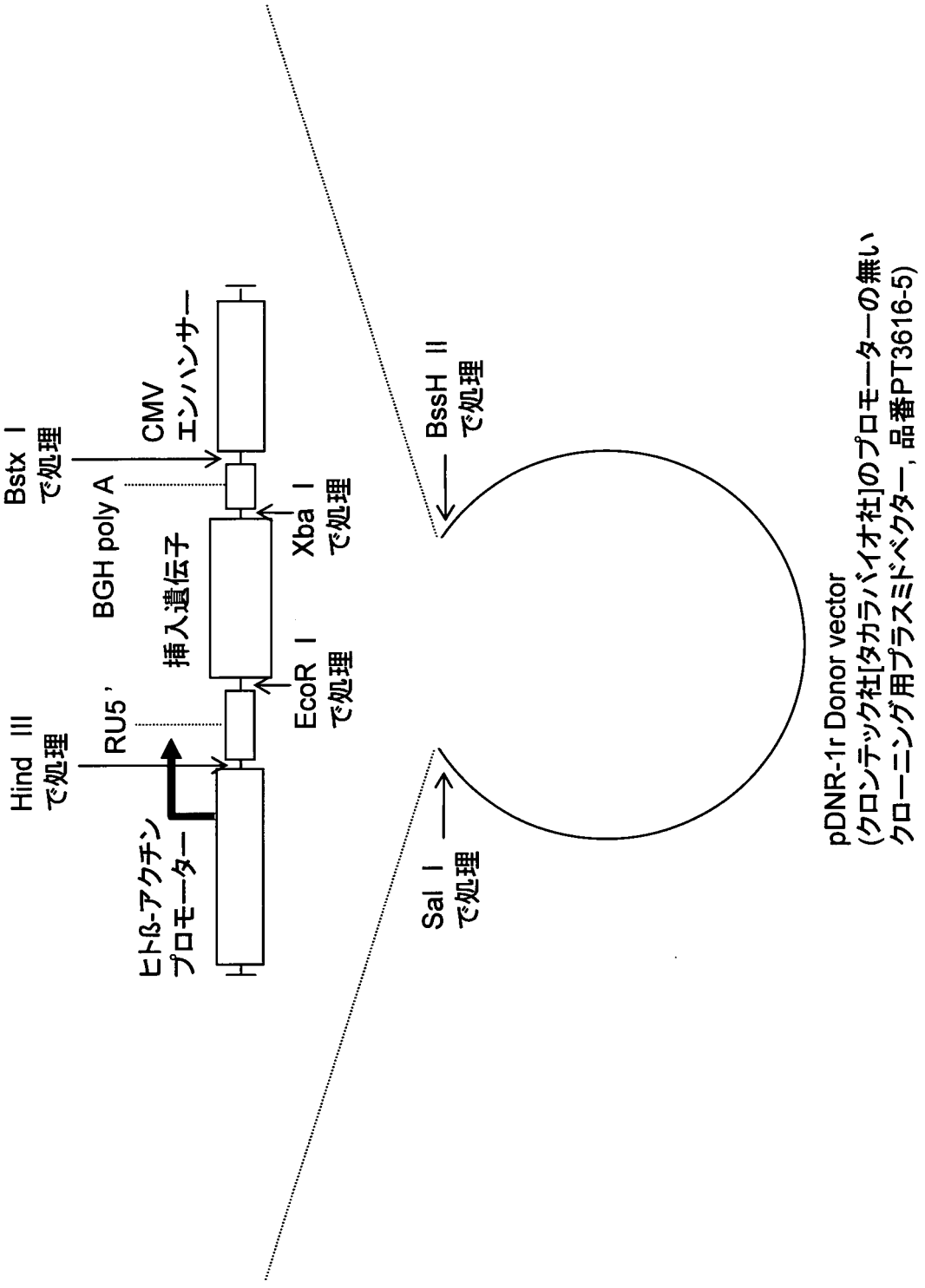


図１３
コンストラクト No.7

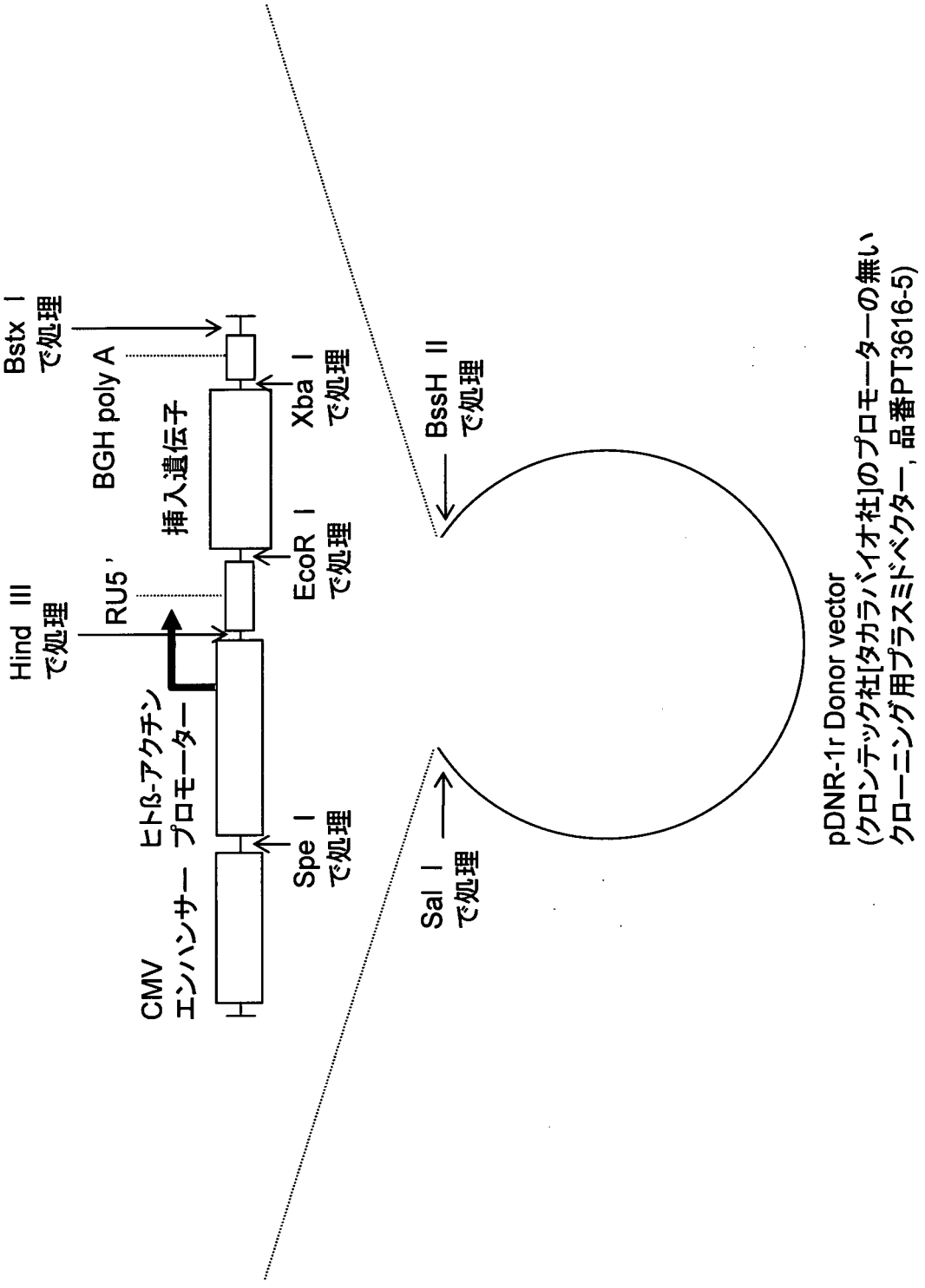


図１４
コンストラクト No.8

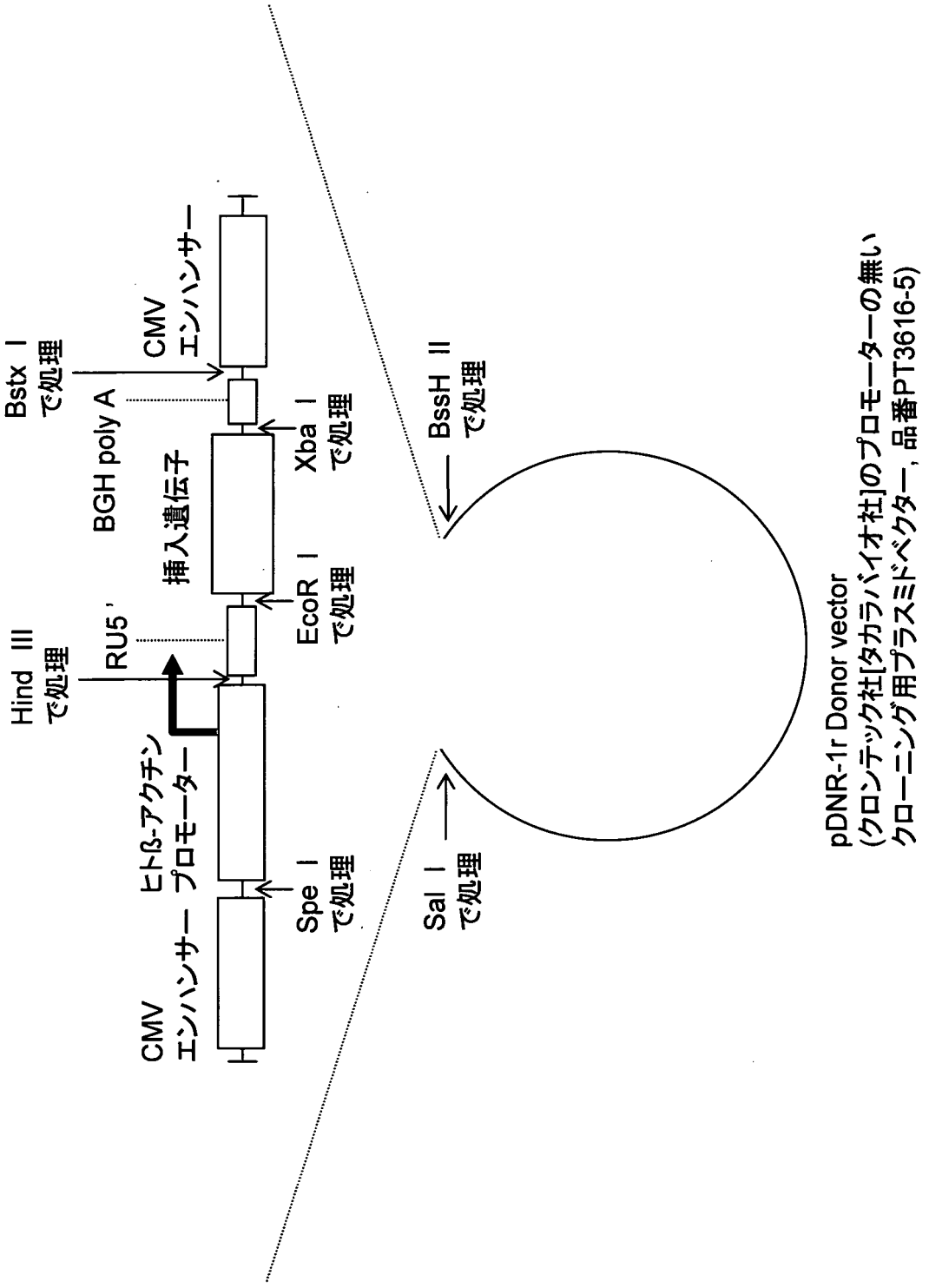


図１５
コンストラクト No.9

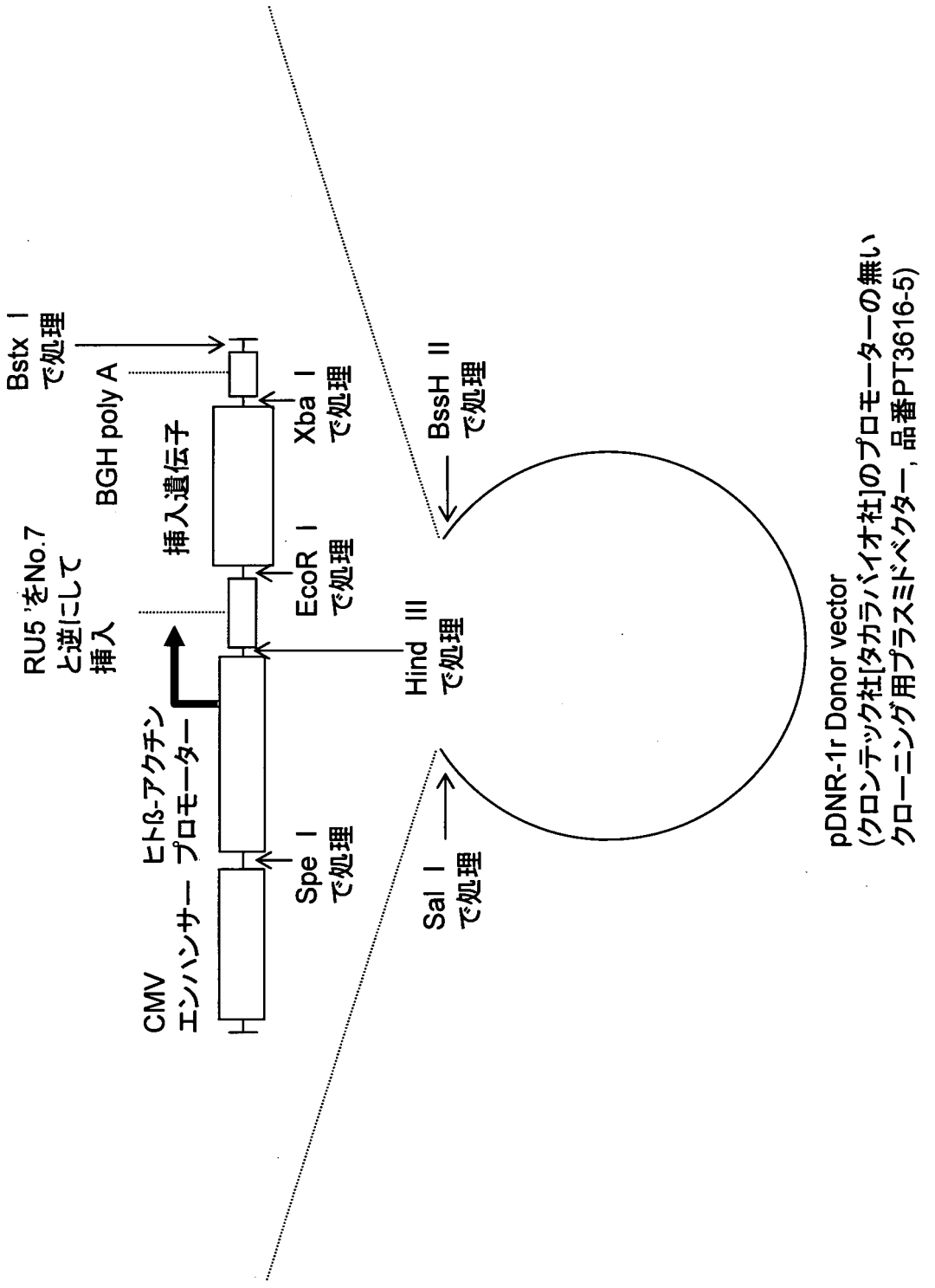


図１６
コンストラクト No.10

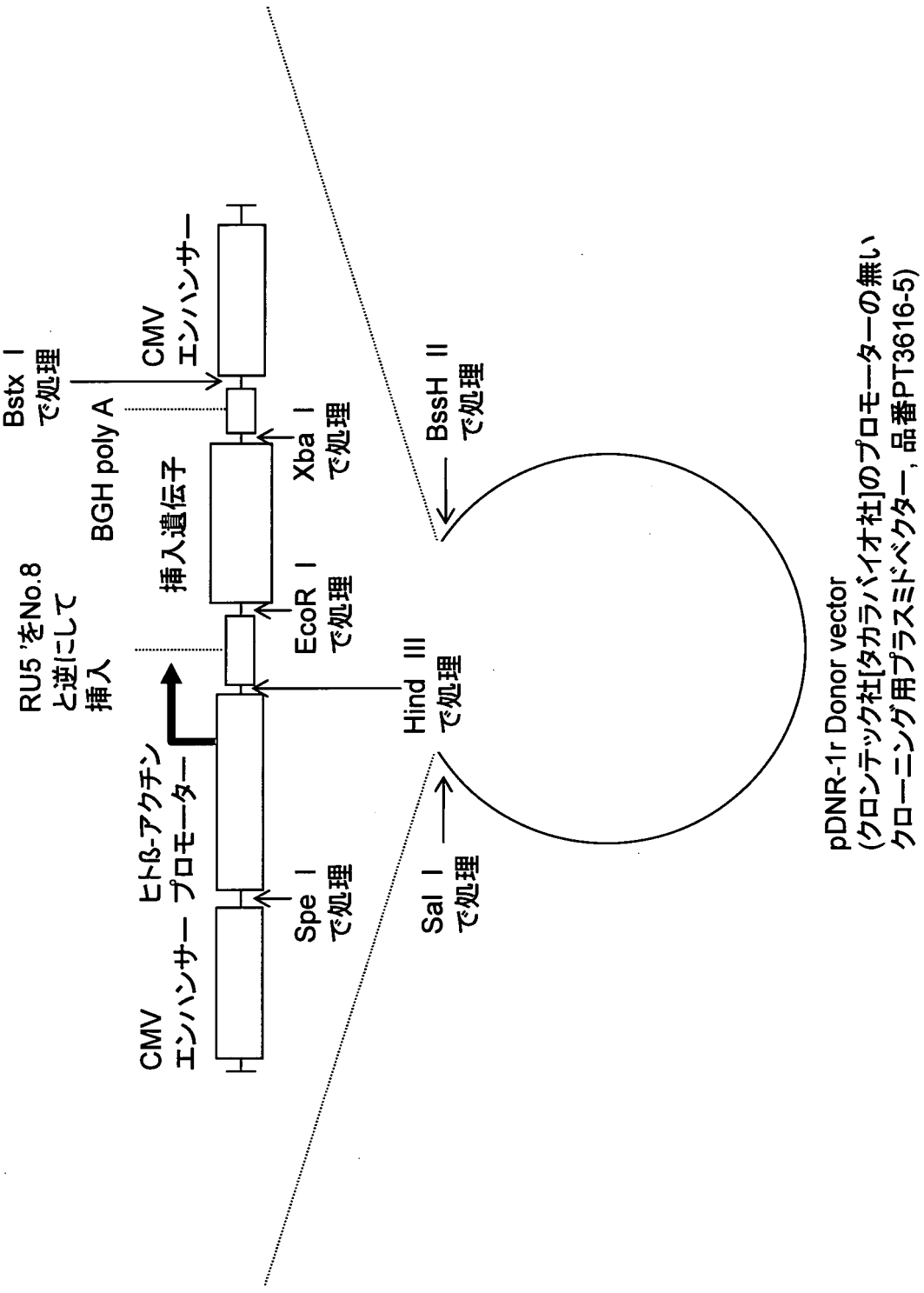


図１７
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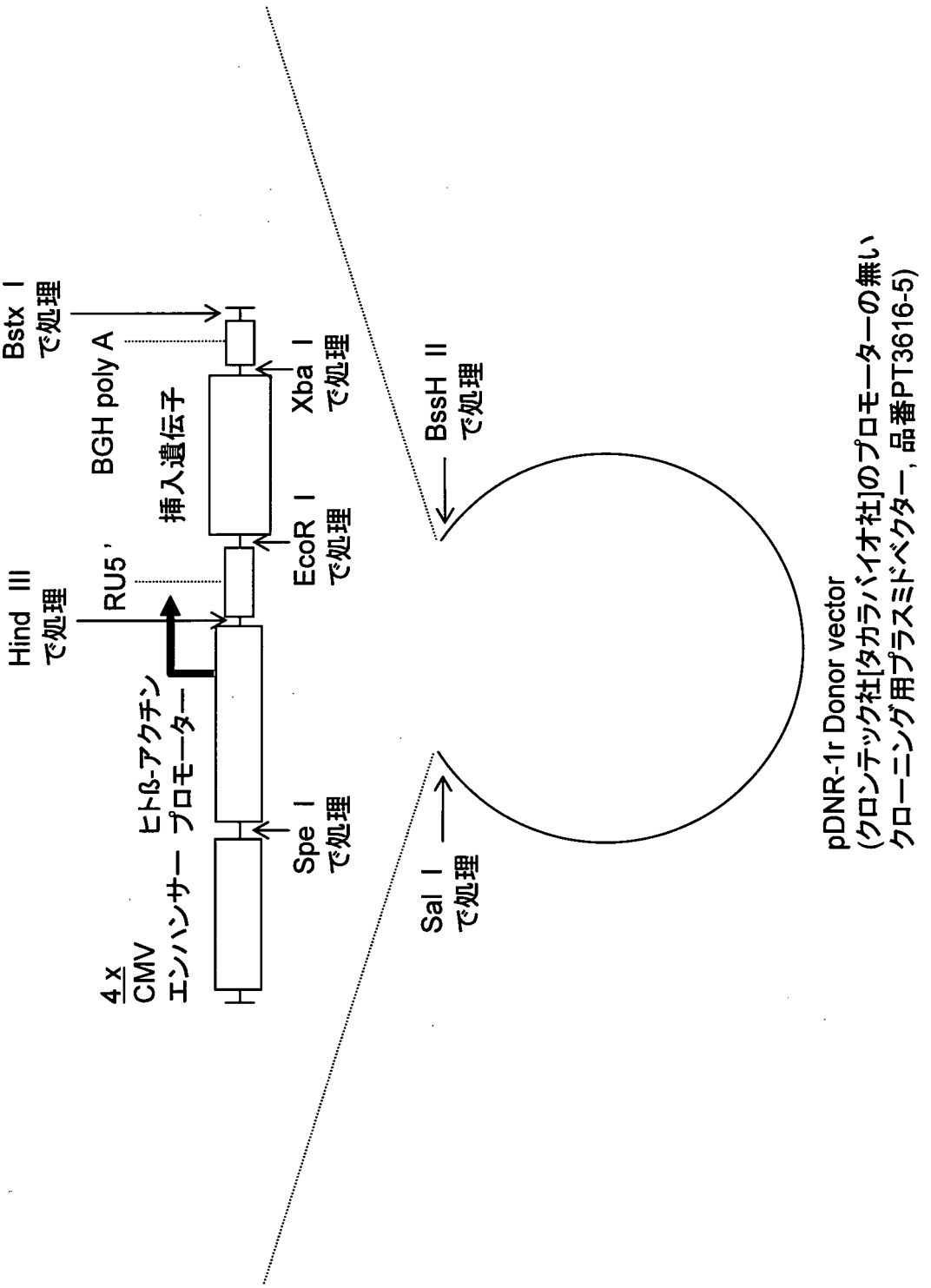


図１８
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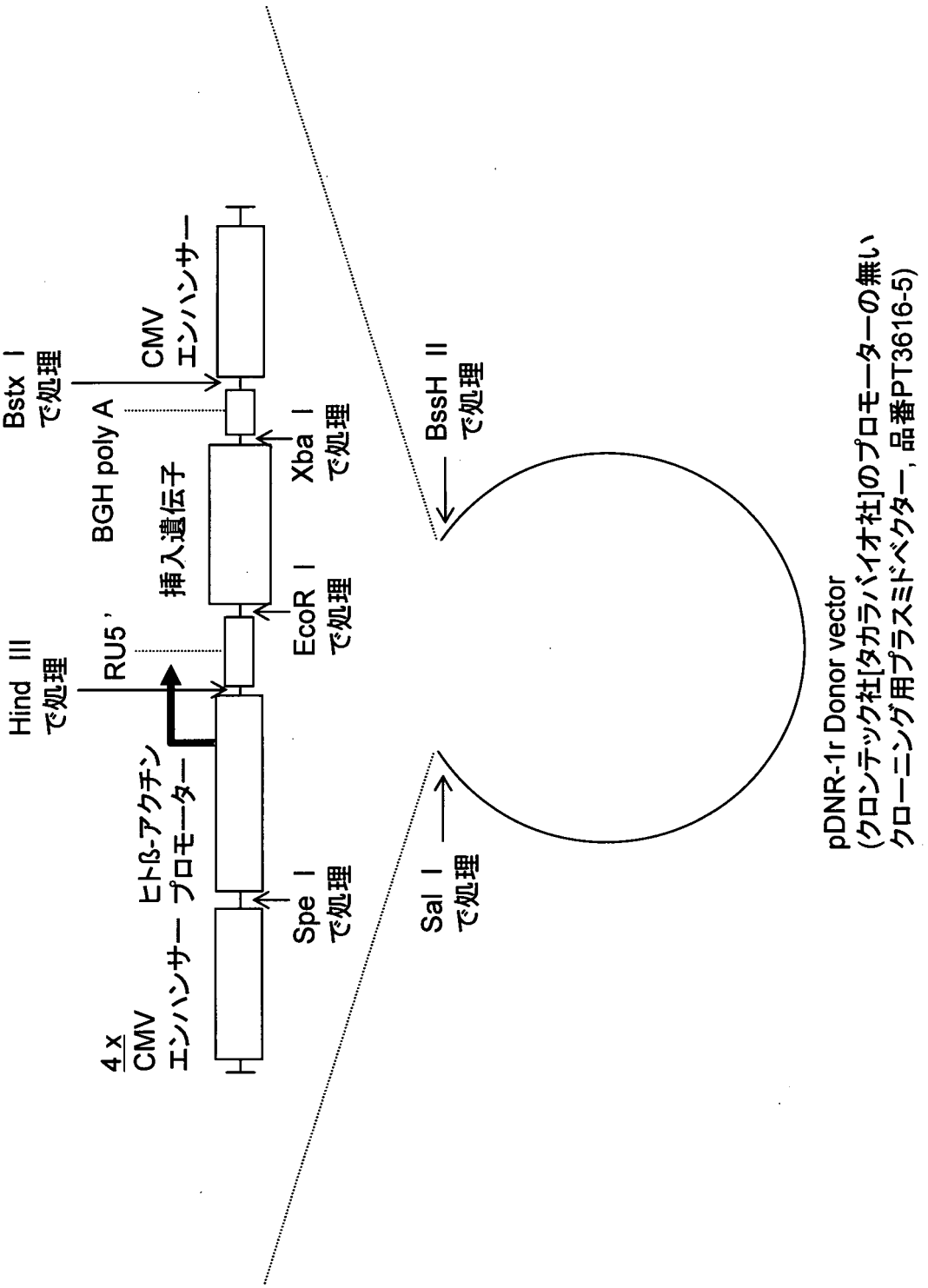


図１９
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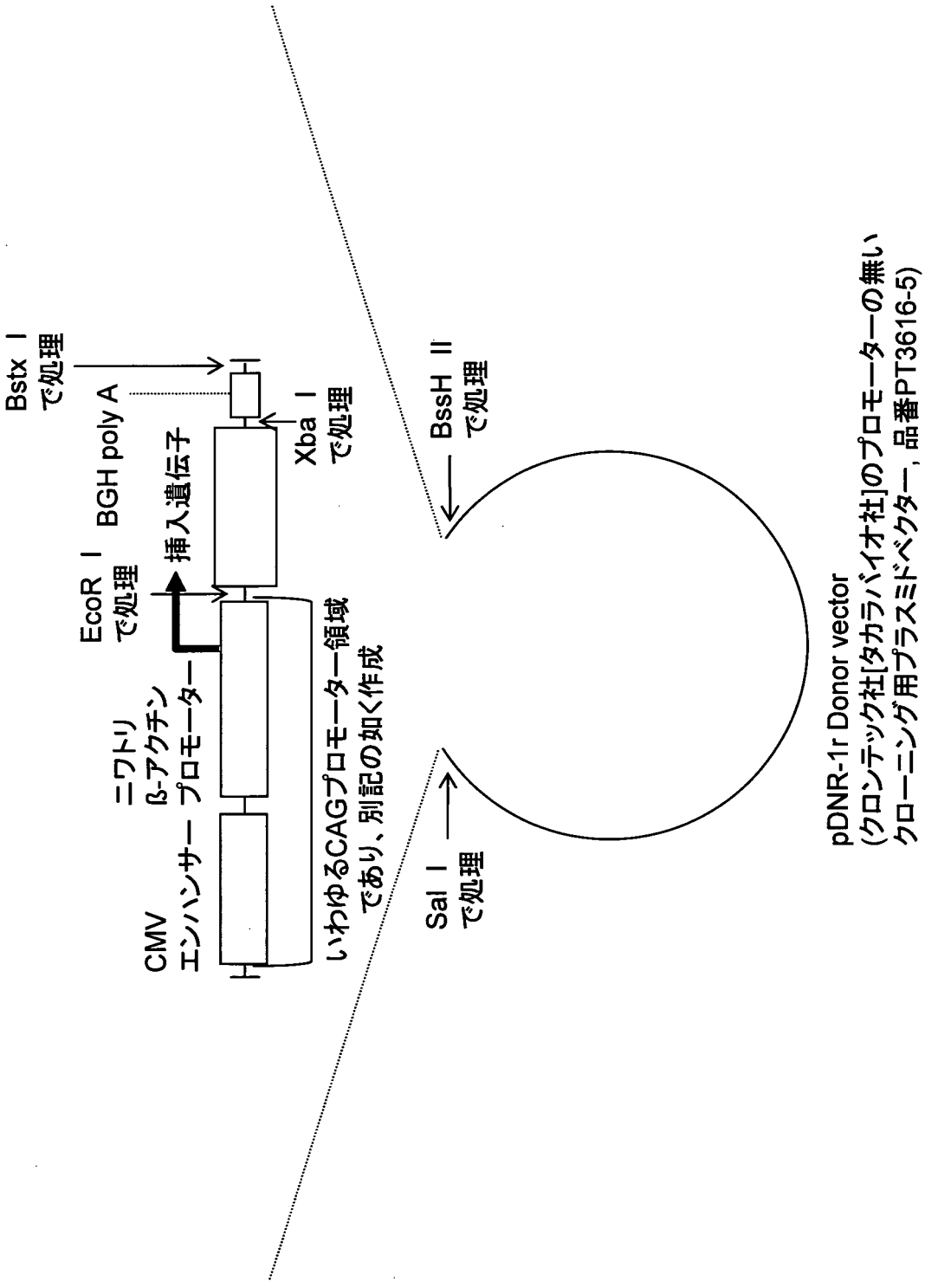


図２０
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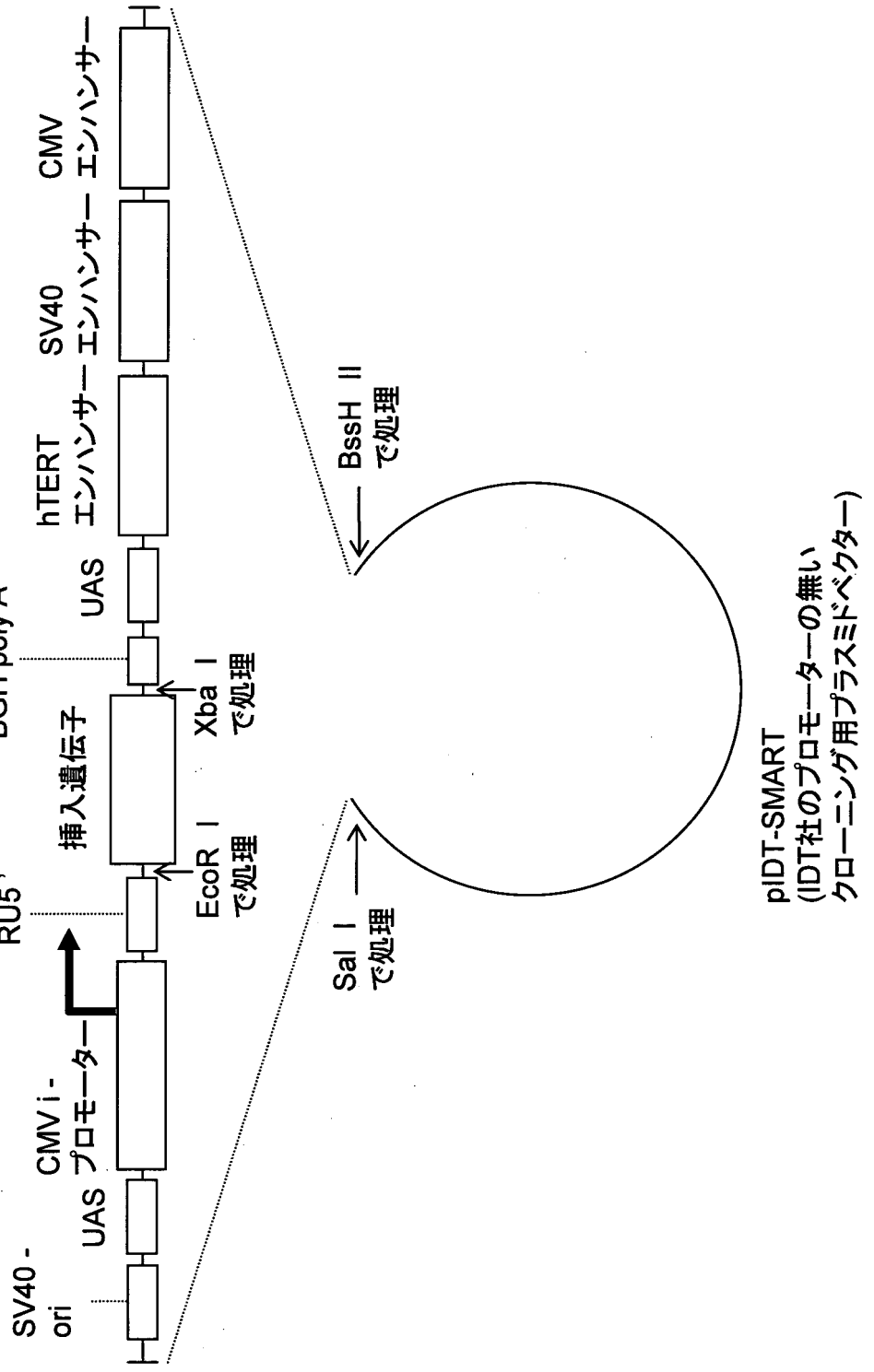


図21

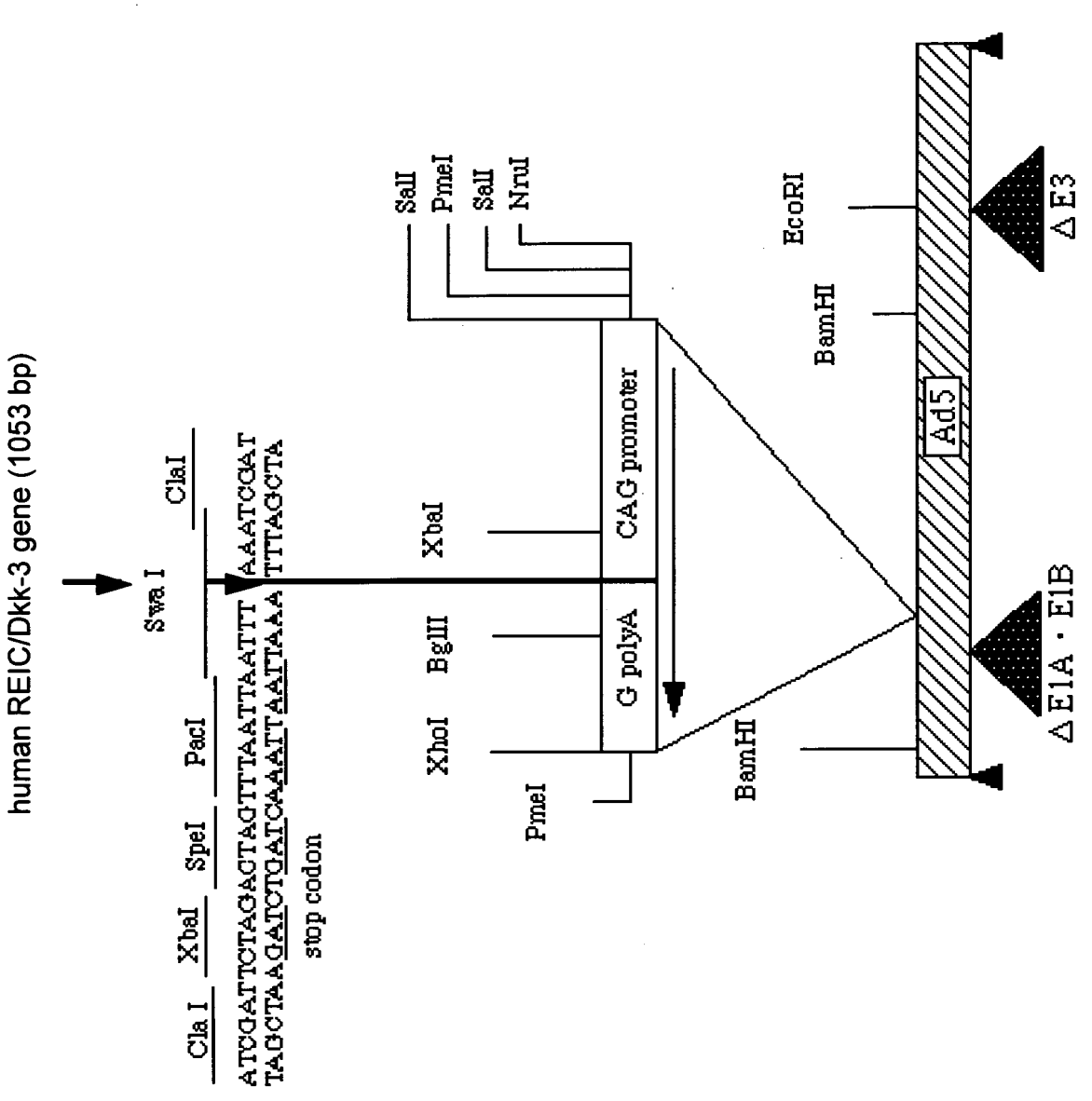

human REIC/Dkk-3 gene (1053 bp)
Swa I
ClaI
Cla I
XbaI
SpeI
PacI
ATCGATTCTAGACTAGTTAATTAATTT
TAGCTAAGATCTGATCAATTAATTAAA
stop codon
XbaI
SalI
PmeI
SalI
NruI
CAG promoter
G polyA
XhoI
BglII
PmeI
BamHI
EcoRI
BamHI
Ad5
ΔE3
ΔE1A・E1B

図２２－１

pDNR-1r Donor vector の全塩基配列

a) アンピシリン耐性
b) ボールド字体中にマルチクローニング部位

GTCGAC: SalI, GCGCGC: BssHII

GCGGCCGCATAACTTCGTATAGCATACATTATACGAAGTTAT**CA**GTCGAC 発現遺伝子をここに挿入する
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

図２２－２

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

図２３

pIDT-SMART vector の全塩基配列

a) カナマイシン耐性
b) ボールド字体中にマルチクローニング部位

GATATC:EcoRV

GGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTC
TTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATT
AGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTA
CACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTCGGAAAAAGAGT
TGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGC
AGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATTTTCTACCGAAGAAAG
GCCCACCCGTGAAGGTGAGCCAGTGAGTTGATTGCAGTCCAGTTACGCTGGAGTCTGAGGCT
CGTCCTGAATGTGTAAAACGACGGCCAGTTTATCTAGTCAGCTTGATTCTAGCTGATCGTGG
ACCGGAAGGTGAGCCAGTGAGTTGATTGCAGTCCAGTTACGCTGGAGTCTGAGGCTCGTCCT
GAATGATAT**ACGCGTCGGAGGGTTGCGTTTGAGACGGGCGACA**GAT 発現遺伝子をここに挿入する
ATCAGTTCTGGACGAGCGAGCTGTCGTCCGGCGGCCGCGATCTTACGGCATTATACGTATGA
TCGGTCCACGATCAGCTAGATTATCTAGTCAGCTTGATGTCATAGCTGTTTCCTGAGGCTCA
ATACTGACCATTTAAATCATACCTGACCTCCATAGCAGAAAGTCAAAAGCCTCCGACCGGAG
GCTTTTGACTTGATCGGCACGTAAGAGGTTCCAACTTTCACCATAATGAAATAAGATCACTA
CCGGGCGTATTTTTTGAGTTATCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGAGCCATAT
TCAACGGGAAACGTCTTGCTTGAAGCCGCGATTAAATTCCAACATGGATGCTGATTTATATG
GGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGG
AAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTAC
AGATGAGATGGTCAGGCTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATT
TTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCAGGGAAAACAGCATTC
CAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCT
GCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACGGCGATCGCGTATTTCGTC
TCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGGTGCGAGTGATTTTGATGACGAG
CGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTCTTGCCATTCTCACC
GGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAAT
TAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATC
CTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGG
TATTGATAATCCTGATATGAATAAATTGCAGTTTCACTTGATGCTCGATGAGTTTTTCTAAT
GAGGACCTAAATGTAATCACCTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTGCTGGCGTTT
TTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGATGCTCAAGTCAGAGGTGGCG
AAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTC
CTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCG
CTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCT

図２４

CMVi プロモーター(hCMV + イントロンプロモーター)領域の塩基配列

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

図２５

BGH polyA(3 x stop ＋ BGH polyA)領域の塩基配列

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

図２６

CMV エンハンサー領域の塩基配列

Tccgcgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgccca
ttgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtca
atgggtggagtatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaa
gtacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatg
accttatgggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggt

図２７

ヒトβ-アクチンプロモーター領域の塩基配列

gttccatgtccttatatggactcatctttgcctattgcgacacacactcagtgaacacctac
tacgcgctgcaaagagccccgcaggcctgaggtgcccccacctcaccactcttcctatttttt
gtgtaaaaatccagcttcttgtcaccacctccaaggaggggggaggaggaggaaggcaggttc
ctctaggctgagccgaatgcccctctgtggtcccacgccactgatcgctgcatgcccaccac
ctgggtacacacagtctgtgattcccggagcagaacggaccctgcccacccggtcttgtgtg
ctactcagtggacagacccaaggcaagaaagggtgacaaggacagggtcttcccaggctggc
tttgagttcctagcaccgccccgcccccaatcctctgtggcacatggagtcttggtccccag
agtcccccagcggcctccagatggtctgggagggcagttcagctgtggctgcgcatagcaga
catacaacggacggtgggcccagacccaggctgtgtagacccagccccccgccccgcagtg
cctaggtcacccactaacgccccaggccttgtcttggctgggcgtgactgttaccctcaaaa
gcaggcagctccagggtaaaaggtgccctgccctgtagagcccaccttccttcccagggctg
cggctgggtaggtttgtagccttcatcacgggccacctccagccactggaccgctggcccct
gccctgtcctggggagtgtggtcctgcgacttctaagtggccgcaagccacctgactccccc
aacaccacactctacctctcaagcccaggtctctccctagtgacccacccagcacatttagc
tagctgagccccacagccagaggtcctcaggccctgctttcagggcagttgctctgaagtcg
gcaaggggagtgactgcctggccactccatgccctccaagagctccttctgcaggagcgta
cagaacccagggccctggcacccgtgcagaccctggcccaccccacctgggcgctcagtgcc
caagagatgtccacacctaggatgtcccgcggtgggtgggggggcccgagagacgggcaggcc
gggggcaggcctggccatgcggggccgaaccgggcactgcccagcgtggggcgcgggggcca
cggcgcgcgcccccagcccccgggcccagcaccccaaggcggccaacgccaaaactctccct
cctcctcttcctcaatctcgctctcgctctttttttttttcgcaaaaggaggggagaggggg
taaaaaaatgctgcactgtgcggcgaagccggtgagtgagcggcgcggggccaatcagcgtg
cgccgttccgaaagttgccttttatggctcgagcggccgcggcggcgccctataaaacccag
cggcgcgacgcgccaccaccgccgagaccgcgtccgccccgcgagcacagagcctcgccttt
gccgatccgccgcccgtcca

図２８

RU5′ 正方向 {HTLV Type 1 long terminal repeat のＲセグメント及びU5配列の一部(R-U5′)}領域の塩基配列

agcttcgaggggctcgcatctctccttcacgcgcccgccgccctacctgaggccgccatcca
cgccggttgagtcgcgttctgccgcctcccgcctgtggtgcctcctgaactgcgtccgccgt
ctaggtaagtttaaagctcaggtcgagaccgggcctttgtccggcgctcccttggagcctac
ctagactcagccggctctccacgctttgcctgaccctgcttgctcaactctacgtctttgtt
tcgttttctgttctgcgccgttacagatccaagccacc

図２９

RU5′ 逆方向 {HTLV Type 1 long terminal repeat のＲセグメント及びU5配列の一部(R-U5′)}領域の塩基配列

ggtggcttggatctgtaacggcgcagaacagaaaacgaaacaaagacgtagagttgagcaag
cagggtcaggcaaagcgtggagagccggctgagtctaggtaggctccaagggagcgccggac
aaaggcccggtctcgacctgagctttaaacttacctagacggcggacgcagttcaggaggca
ccacaggcgggaggcggcagaacgcgactcaaccggcgtggatggcggcctcaggtagggcg
gcgggcgcgtgaaggagagatgcgagcccctcgaagct

図３０

4 x CMV エンハンサー領域の塩基配列

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

図３１

CAGプロモーター領域の塩基配列

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

図３２

2IRES インサート領域の塩基配列

(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

図３３

SV40ori-UAS-CMVi-RU5′領域の塩基配列

(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

図３４

3 x stop-BGH-polyA-UAS-hTERT エンハンサー+ SV40 エンハンサー+ CMV エンハンサー領域の塩基配列

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

TCTAGAGC*tagatgactaac*GTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGC (1)
CAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCAC
TGTCCTTTCCT*AATAAA*ATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTC
TGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCT
GGGGATGCGGTGGGCTCTATGG**cggagtactgtcctccg**cttcccacgtggcggagggactg (2)
gggacccgggcacccgtcctgcccttcaccttccagctccgcctcctccgcgcggaccccg
ccccgtcccgacccctcccgggtccccggcccagcccccctccgggccctcccagcccctccc
cttcctttccgcggccccgccctctcctcgcggcgcgagtttTGGAAAGTCCCCAGGCTCCC (3)
CAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCC
CCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAACCATAGT
CCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCC
ATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTC
CAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCAAGGCTTTTGCAAAAAGCTCcgttacataac (4)
ttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatg
acgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtattt
acggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattg
acgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggacttt
cctacttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggca
gtacatcaatgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattg
acgtcaatgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaac
tccgccccattgacgcaaatgggcggtaggcgtgTTGGCGCGC

図３５－１

コンストラクト No.14 ベクターの全塩基配列

GGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTC
TTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATT
AGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTA
CACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTCGGAAAAAGAGT
TGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGC
AGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATTTTCTACCGAAGAAAG
GCCCACCCGTGAAGGTGAGCCAGTGAGTTGATTGCAGTCCAGTTACGCTGGAGTCTGAGGCT
CGTCCTGAATGTGTAAAACGACGGCCAGTTTATCTAGTCAGCTTGATTCTAGCTGATCGTGG
ACCGGAAGGTGAGCCAGTGAGTTGATTGCAGTCCAGTTACGCTGGAGTCTGAGGCTCGTCCT
GAATGATAT**ACGCGTCGGAGGGTTGCGTTTGAGACGGGCGACAGAT**ACGCGTCGACGTCGGC
CATAaatttttgcaaaagcttggcctccaaaaaagcctcctcactacttctggaatagct (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
gatcccccagccagcggtccgcaacccttgccgcatccacgaaactttgcccatagcagcgg
gcgggcactttgcactggaacttacaacacccgagcaaggacgcgactctcccgacgcgggg
aggctattctgcccatttggggacacttccccgccgctgccaggacccgcttctctgaaagg
ctctccttgcagctgcttagacgctggatttttttcgggtagtggaaaaccagcagcctccc
gcgCCGCTCGAGCGGAAAAGGCCTTTTGCTCTAGAGCtagatgactaacGTTTAAACCCGCT

図３５－２

GATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCT
TCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATC
GCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGG
AGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG**cggagtactgt**
cctccgcttcccacgtggcggagggactggggacccgggcacccgtcctgcccttcaccttt (6)
ccagctccgcctcctccgcgcggaccccgccccgtcccgacccctcccgggtccccggccca
gccccctccgggccctcccagccccctccccttcctttccgcggccccgccctctcctcgcgg
cgcgagtttTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCA (7)
ATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGC
ATCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAAC
TCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGG
CCGAGGCCGCCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCAA
GGCTTTTGCAAAAAGCTCcgttacataacttacggtaaatggcccgcctggctgaccgccca
acgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggact
ttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggcagtacatcaagt
gtatcatatgccaagtacgccccctattgacgtcaatgacggtaaatggcccgcctggcatt
atgcccagtacatgaccttatgggactttcctacttggcagtacatctacgtattagtcatc
gctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatagcggtttgactc
acggggatttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatc
aacgggactttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgt
gTTGGCGCGCCCAA**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

図３６

コンストラクト No.15

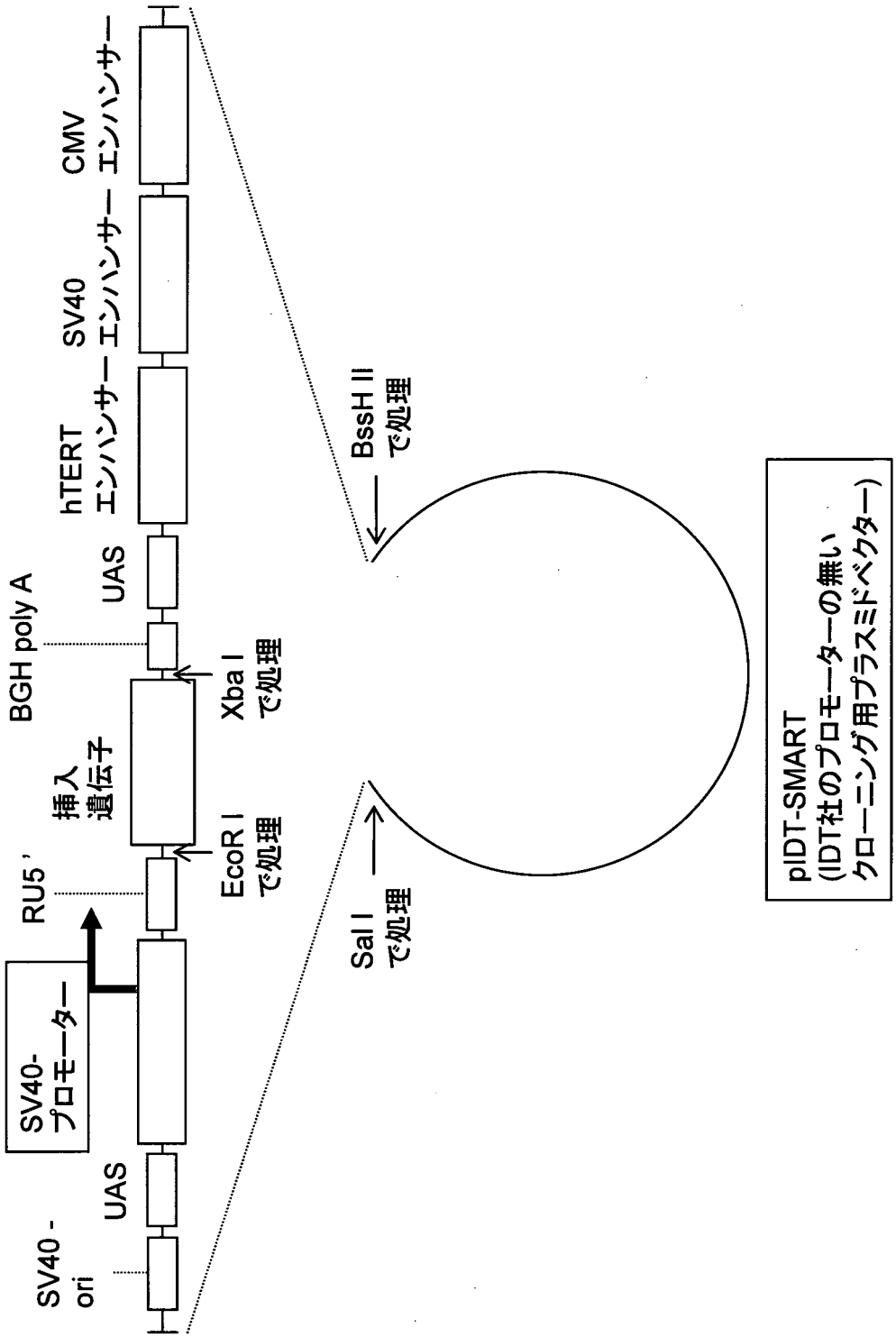


図３７

コンストラクト No.16

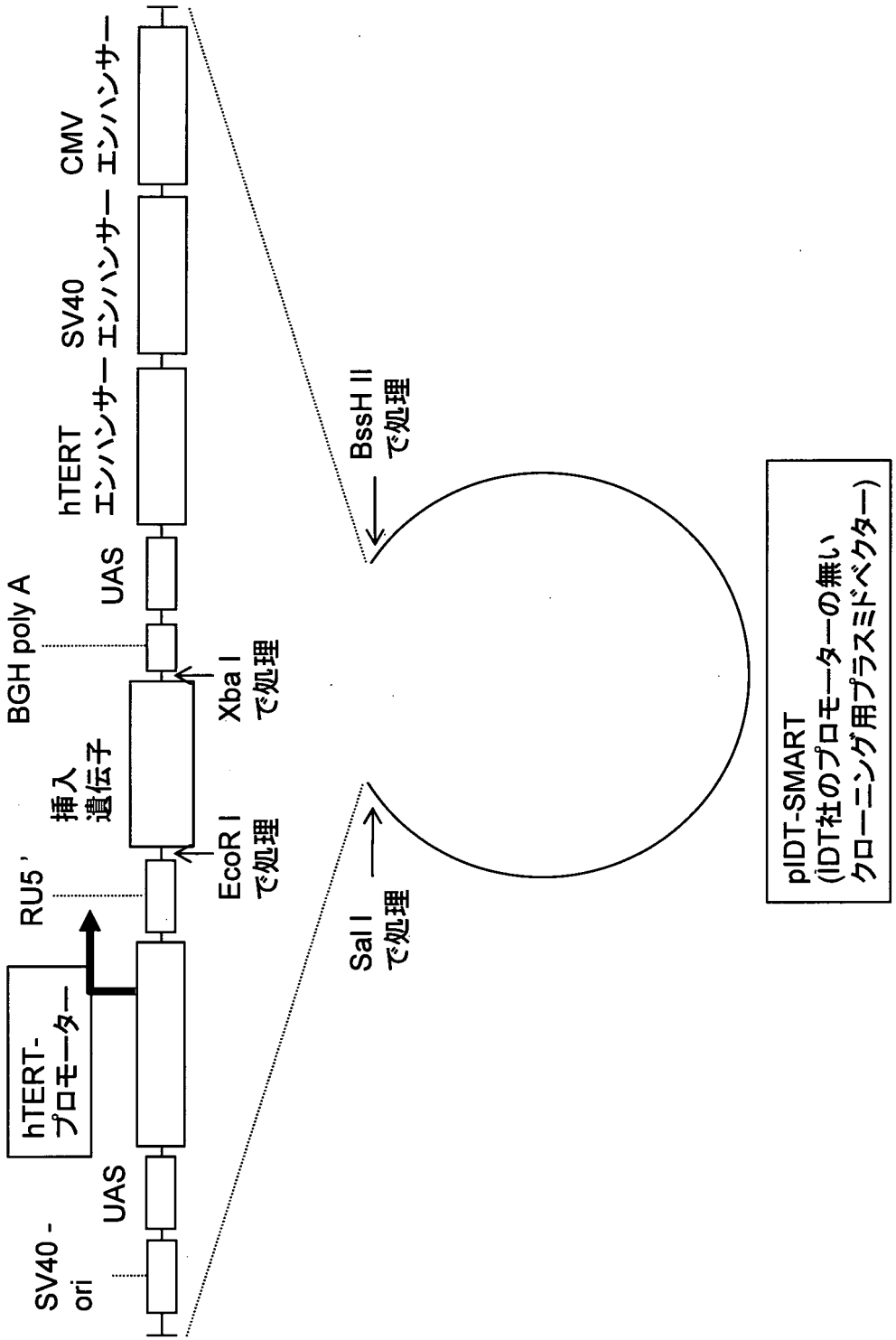


図３８

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)
catccacgctgttttgacctccatagaagacaccgggaccgatccagcctccgcggccggga
acggtgcattggaacgcggattccccgtgccaagagtgacgtaagtaccgcctatagactct
ataggcacacccctttggctcttatccatcaattaatacgactcactatagggagacagact
gttcctttcctgggtcttttctg
gcttcgaggggctcgcatctctccttcacgcgcccgccgccctacctgaggccgccatccac (4)
gccggttgagtcgcgttctgccgcctcccgcctgtggtgcctcctgaactgcgtccgccgtc
taggtaagtttaaagctcaggtcgagaccgggcctttgtccggcgctcccttggagcctacc
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cgttttctgttctgcgccgttacagatccaagccacc CCGG/AATTCCGG

図３９

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

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

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cgttttctgttctgcgccgttacagatccaagccacc CCGG/AATTCCGG

図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
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acc cgc gcc gag gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc
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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

図４１－１

#14-GFP

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

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

ACGCGTCGACGTCGGCCATA

aatttttgcaaaagccttggcctccaaaaaagcctcctcactacttctggaatagctcaga (1)
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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
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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

図４１－２


ccctaattccagcgagaggcagagggagcgagcgggcggccggctagggtggaagagccggg (3)
cgagcagagctgcgctgcgggcgtcctgggaagggagatccggagcgaatagggggcttcgc
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図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

aatttttgcaaaagccttggcctccaaaaaagcctcctcactacttctggaatagctcaga (1)
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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
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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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図４２－２

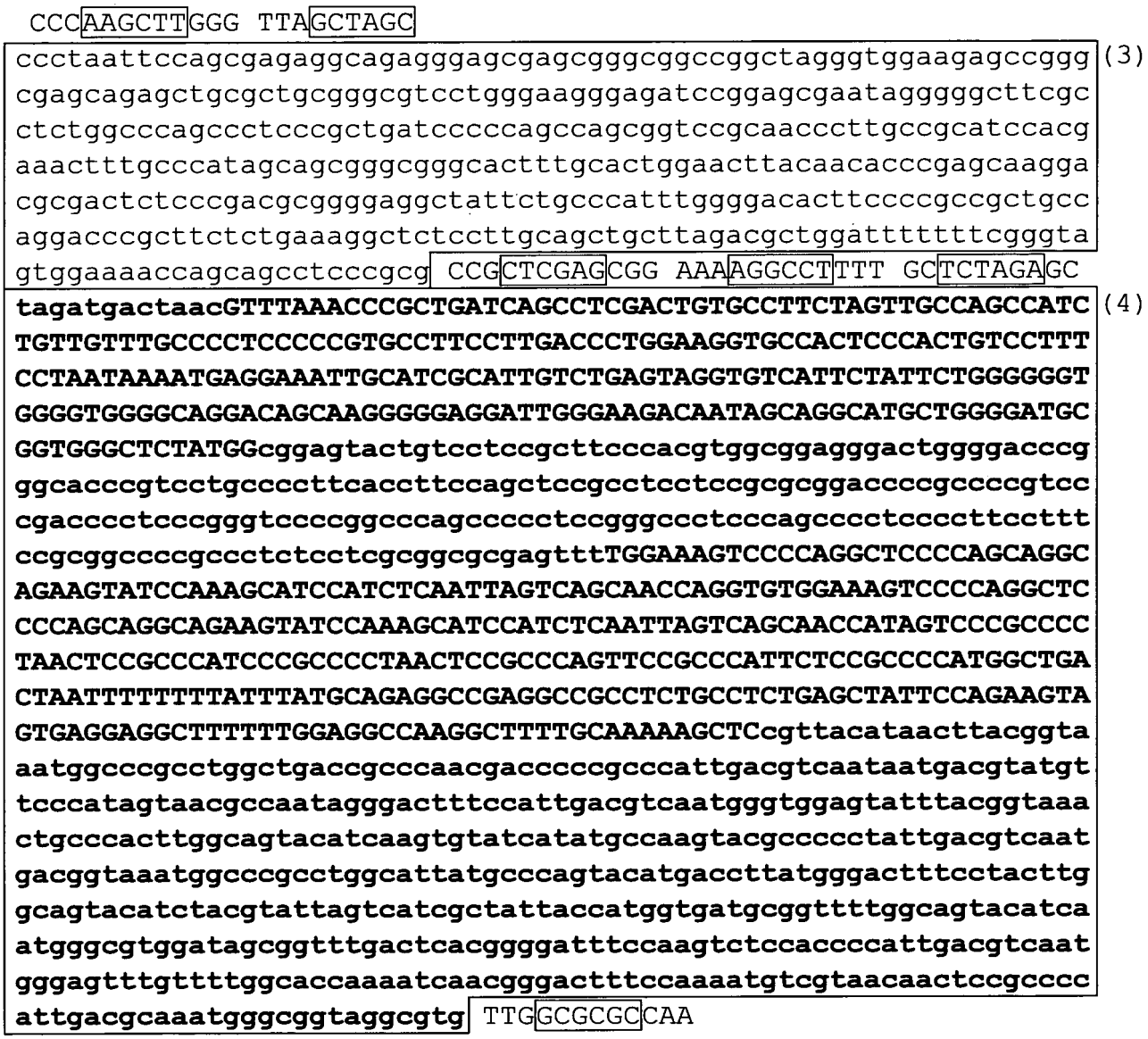

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図４３－１

#16-GFP

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

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

ACGCGTCGACGTCGGCCATA

aatttttgcaaaagccttggcctccaaaaaagcctcctcactacttctggaatagctcaga (1)
ggccgaggcggcctcggcctctgcataaataaaaaaaattagtcagccttggggcggagaaa
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cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag cgc
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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
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図４３－２

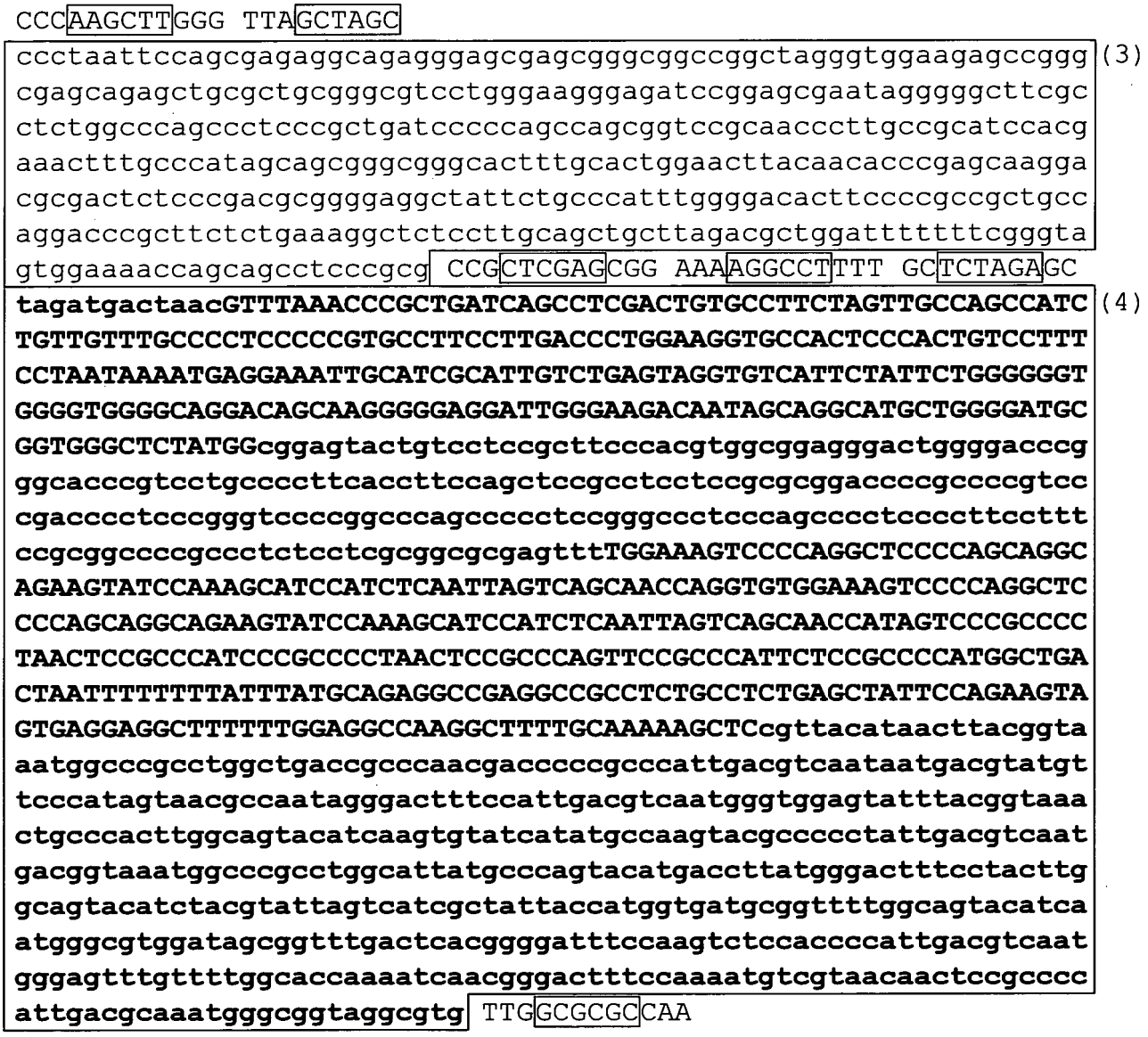

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図44

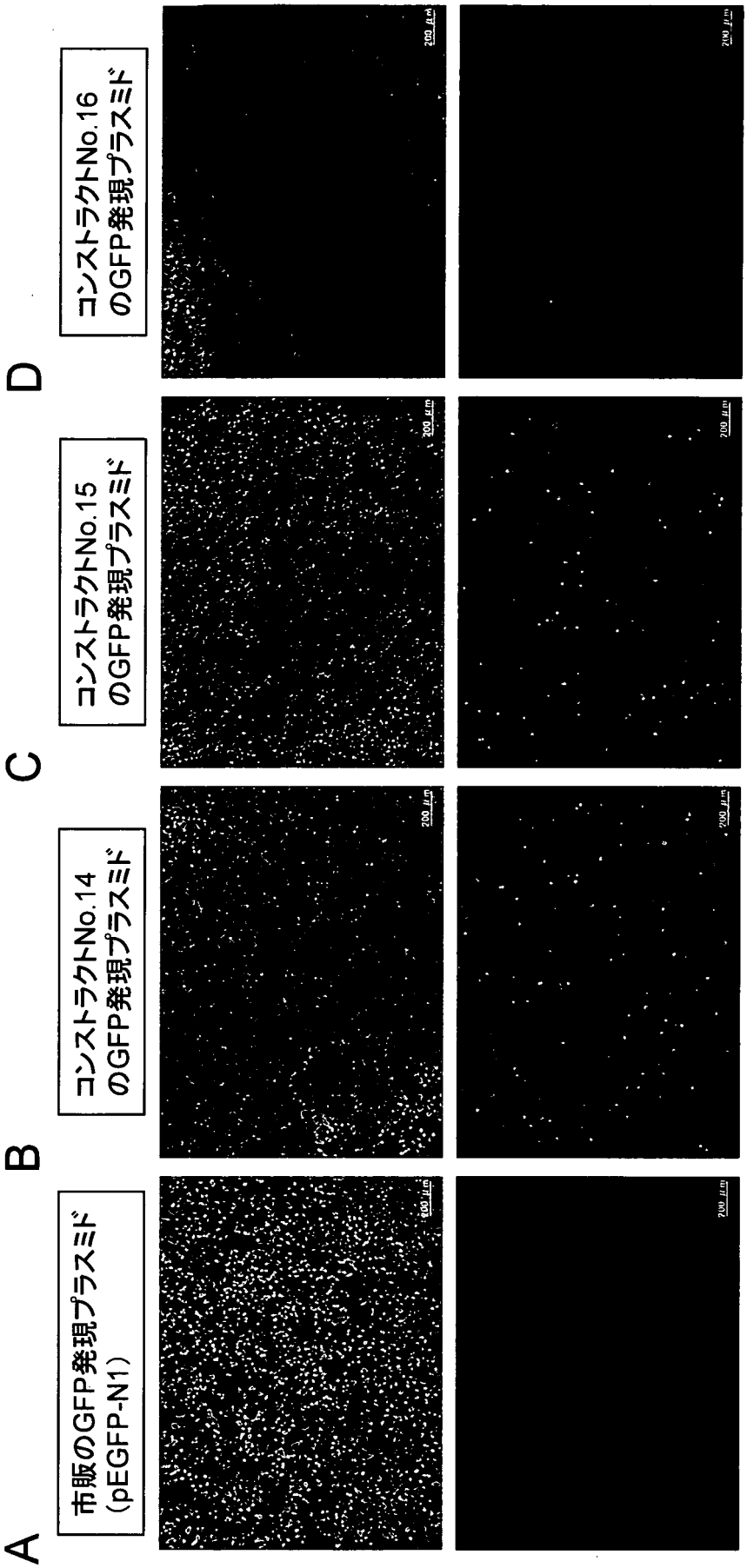

A
市販のGFP発現プラスミド
(pEGFP-N1)
B
コンストラクトNo.14
のGFP発現プラスミド
C
コンストラクトNo.15
のGFP発現プラスミド
D
コンストラクトNo.16
のGFP発現プラスミド

図４５

EPO-6His 遺伝子の配列

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

図４６

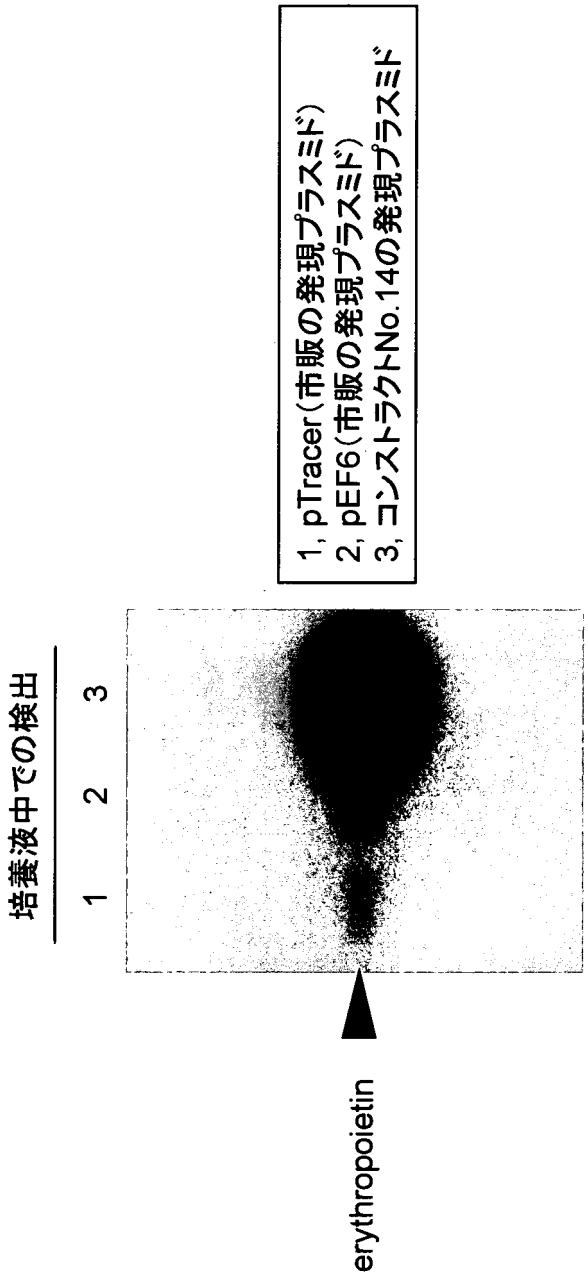

1, pTracer(市販の発現プラスミド)
2, pEF6(市販の発現プラスミド)
3, コンストラクトNo.14の発現プラスミド
培養液中での検出
1
2
3
erythropoietin

図４７Ａ

ヒトの IgG (light chain)**遺伝子の配列**

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

G/AATTCCGG CCG**CTCGAG**CGG 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 GC**T/CTAGAGC
(2)

図４７Ｂ

ヒトの IgG (heavy chain)遺伝子の配列

(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

図４８

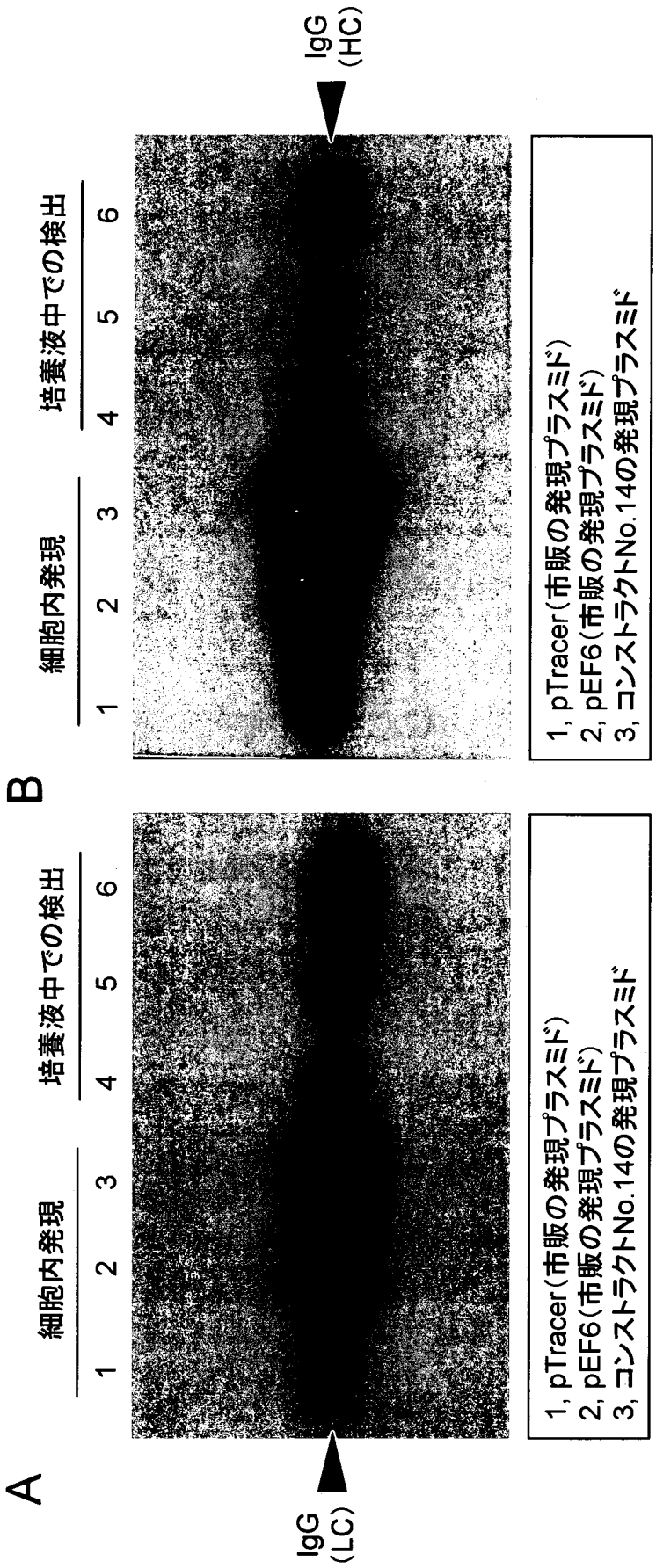

A
細胞内発現
培養液中での検出
1 2 3 4 5 6
IgG (LC)
1, pTracer(市販の発現プラスミド)
2, pEF6(市販の発現プラスミド)
3, コンストラクトNo.14の発現プラスミド
B
細胞内発現
培養液中での検出
1 2 3 4 5 6
IgG (HC)
1, pTracer(市販の発現プラスミド)
2, pEF6(市販の発現プラスミド)
3, コンストラクトNo.14の発現プラスミド

図４９

コンストラクト No.17

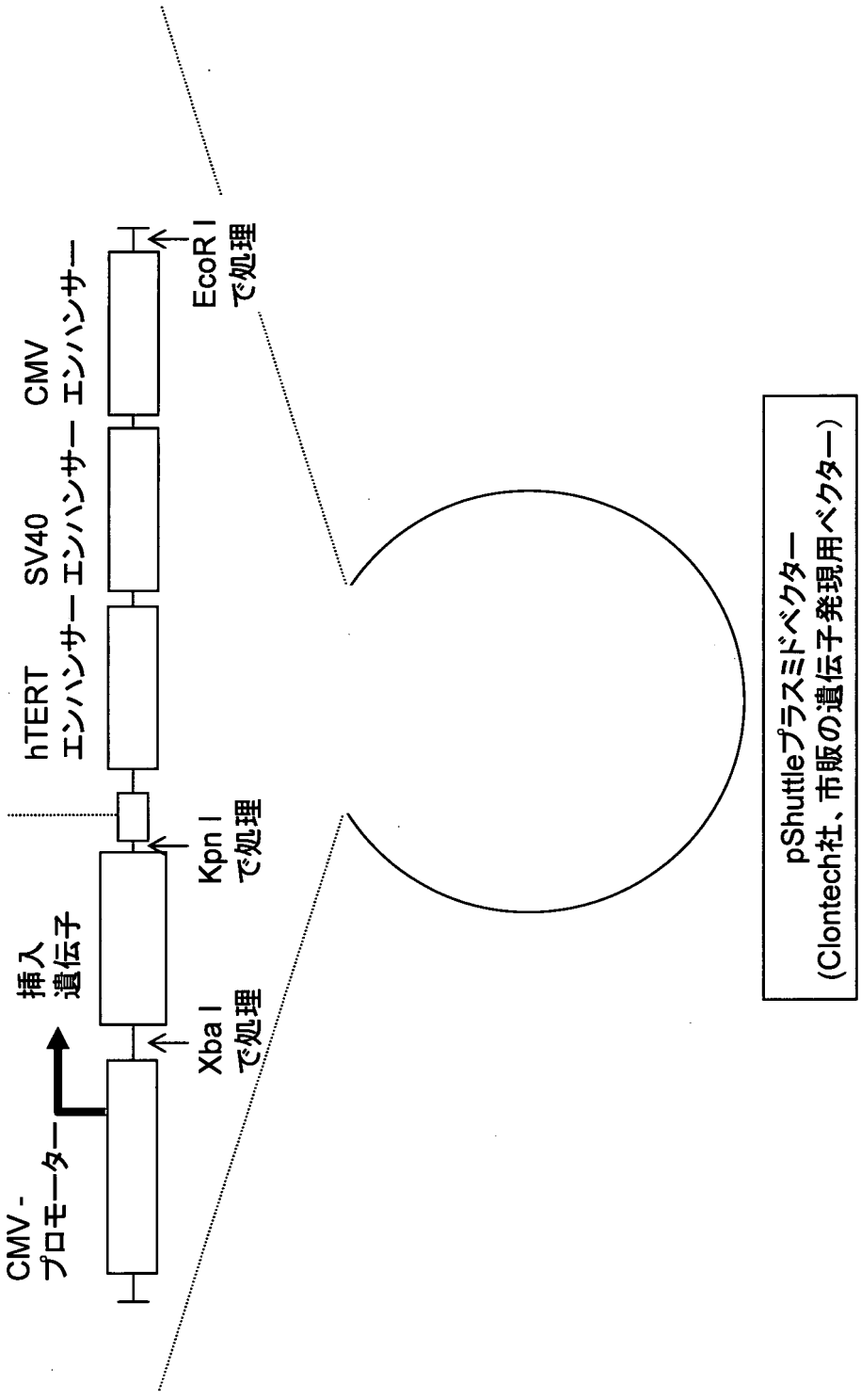


図５０

pshuttle- REIC-TSC

(1) (2)
XbaI-REIC-KpnI-3xenh-EcoRI

T/CTAGAGCacc**atg**cagcggcttggggccaccctgctgtgcctgctgctggcggcggcggt(1)
ccccacggcccccgcgcccgctccgacggcgacctcggctccagtcaagcccggcccggctc
tcagctacccgcaggaggaggccaccctcaatgagatgttccgcgaggttgaggaactgatg
gaggacacgcagcacaaattgcgcagcgcggtggaagagatggaggcagaagaagctgctgc
taaagcatcatcagaagtgaacctggcaaacttacctcccagctatcacaatgagaccaaca
cagacacgaaggttggaaataataccatccatgtgcaccgagaaattcacaagataaccaac
aaccagactggacaaatggtcttttcagagacagttatcacatctgtgggagacgaagaagg
cagaaggagccacgagtgcatcatcgacgaggactgtgggcccagcatgtactgccagtttg
ccagcttccagtacacctgccagccatgccggggccagaggatgctctgcacccgggacagt
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gccagccggcttctggacctcatcacctgggagctagagcctgatggagccttggaccgatg
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gagtatgaagttggcagcttcatggaggaggtgcgccaggagctggaggacctggagaggag
cctgactgaagagatggcgctgggggagcctgcggctgccgccgctgcactgctgggagggg
aagagatt**tag**GG**GGTAC/C**CCGGCtagatgactaacGTTTAAACCCGCTGATCAGCCTCGA
CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTG
GAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAG
TAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAG
ACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG**cggagtactgtcctccg**cttccc(2)
acgtggcggagggactggggacccgggcacccgtcctgccccttcaccttccagctccgcct
cctccgcgcggaccccgccccgtcccgacccctcccgggtccccggcccagcccccctccggg
ccctcccagcccctcccccttcctttccgcggccccgccctctcctcgcggcgcgagtttTGG
AAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAA
CCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAAT
TAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTC
CGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCT
CTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCAAGGCTTTTGCAAA
AAGCTCcgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcc
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caatgggtggagtatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgcc
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aagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggactttc
caaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgTTG**CCGG/AAT**
TC

図５１

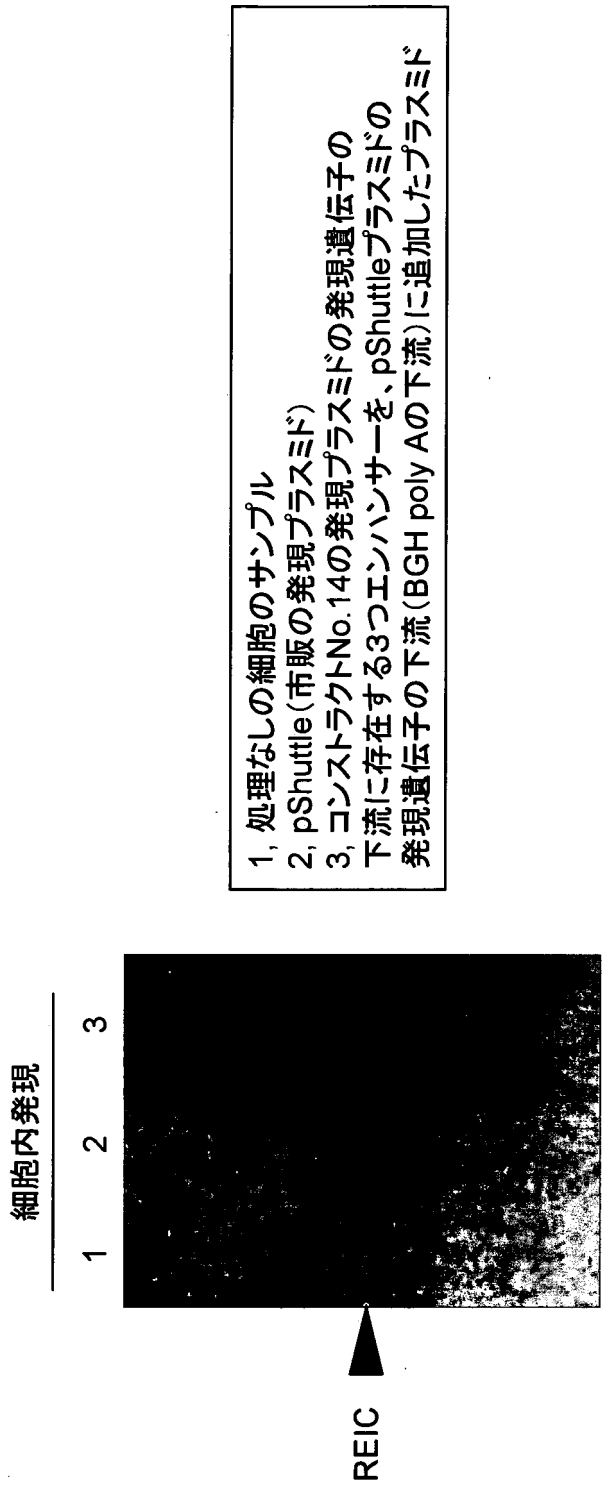

1, 処理なしの細胞のサンプル
2, pShuttle（市販の発現プラスミド）
3, コンストラクトNo.14の発現プラスミドの発現遺伝子の
下流に存在する3つエンハンサーを、pShuttleプラスミドの
発現遺伝子の下流（BGH poly Aの下流）に追加したプラスミド
細胞内発現
1
2
3
REIC

図５２

atgttcatgccttcttcttttcctacagctcctgggcaacgtgctggttattgtgctgt
ctcatcattttggcaaagaattcgcccttcaccatgcccctcaacgtgaacttcaccaac
aggaactatgacctcgactacgactccgtacagccctatttcatctgcgacgaggaagag
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gacatggtgaaccagagcttcatctgcgatcctgacgacgagaccttcatcaagaacatc
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tccccacgggccagccctgagcccctagtgctgcatgaggagacaccgcccaccaccagc
agcgactctgaagaagagcaagaagatgaggaagaaattgatgtggtgtctgtggagaag
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cctccgcacagcccactggtcctcaagaggtgccacgtctccactcaccagcacaactac
gccgcacccccctccacaaggaaggactatccagctgccaagagggccaagttggacagt
ggcagggtcctgaagcagatcagcaacaaccgcaagtgctccagccccaggtcctcagac
acggaggaaaacgacaagaggcggacacacaacgtcttggaacgtcagaggaggaacgag
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gcccccaaggtagtgatcctcaaaaaagccaccgcctacatcctgtccattcaagcagac
gagcacaagctcacctctgaaaaggacttattgaggaaacgacgagaacagttgaaacac
aaactcgaacagcttcgaaactctggtgcataa

図５３

GTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCC
CTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATG
AGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAG
GACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTAT
GGCGGAGTACTGTCCTCCGCTTCCCACGTGGCGGAGGGACTGGTCCTCCGCTTCCCACGTGG
CGGAGGGACTGGGGACCCGGGCACCCGTCCTGCCCCTTCACCTTCCAGCTCCGCCTCCTCCG
CGCGGACCCCGCCCCGTCCCGACCCCTCCCGGGTCCCCGGCCCAGCCCCCTCCGGGCCCTCC
CAGCCCCTCCCCTTCCTTTCCGCGGCCCCGCCCTCTCCTCGCGGCGCGAGTTTTGGAAAGTC
CCCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCAGCAACCAGGT
GTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATCCAAAGCATCCATCTCAATTAGTCA
GCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCA
TTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCT
CTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCAAGGCTTTTGCAAAAAGCTC
CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGA
CGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGG
GTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTAC
GCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCT
TATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATG
CGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCT
CCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAAT
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図５４

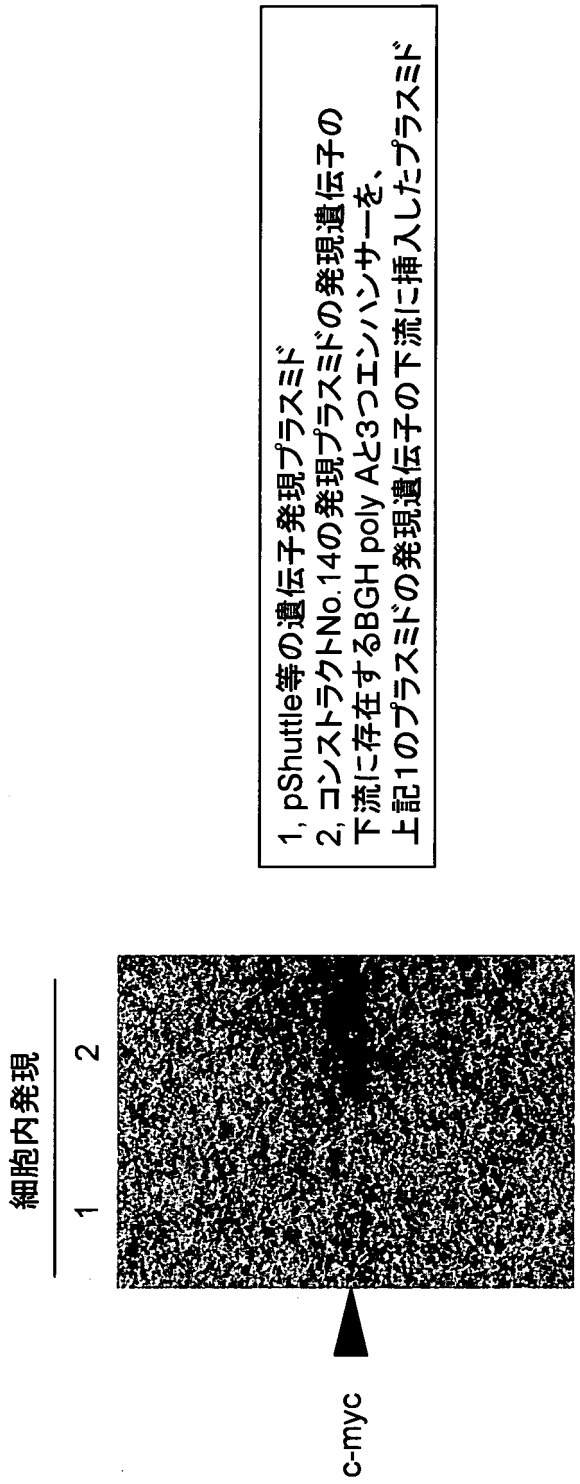

1, pShuttle等の遺伝子発現プラスミド
2, コンストラクトNo.14の発現プラスミドの発現遺伝子の
下流に存在するBGH poly Aと3つエンハンサーを、
上記1のプラスミドの発現遺伝子の下流に挿入したプラスミド
細胞内発現
1
2
c-myc

図５５

コンストラクト No.18

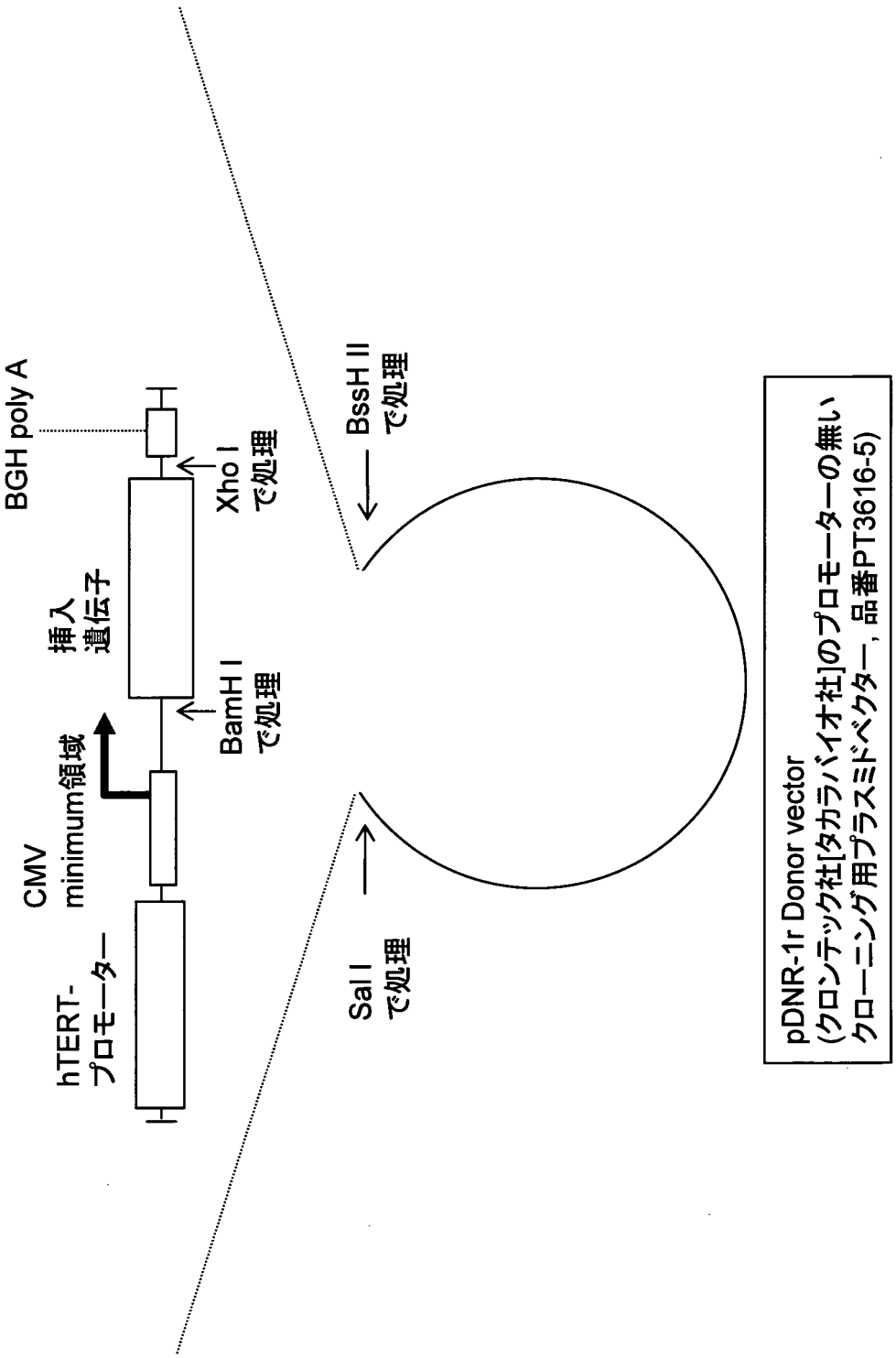


図５６

コンストラクト No.19

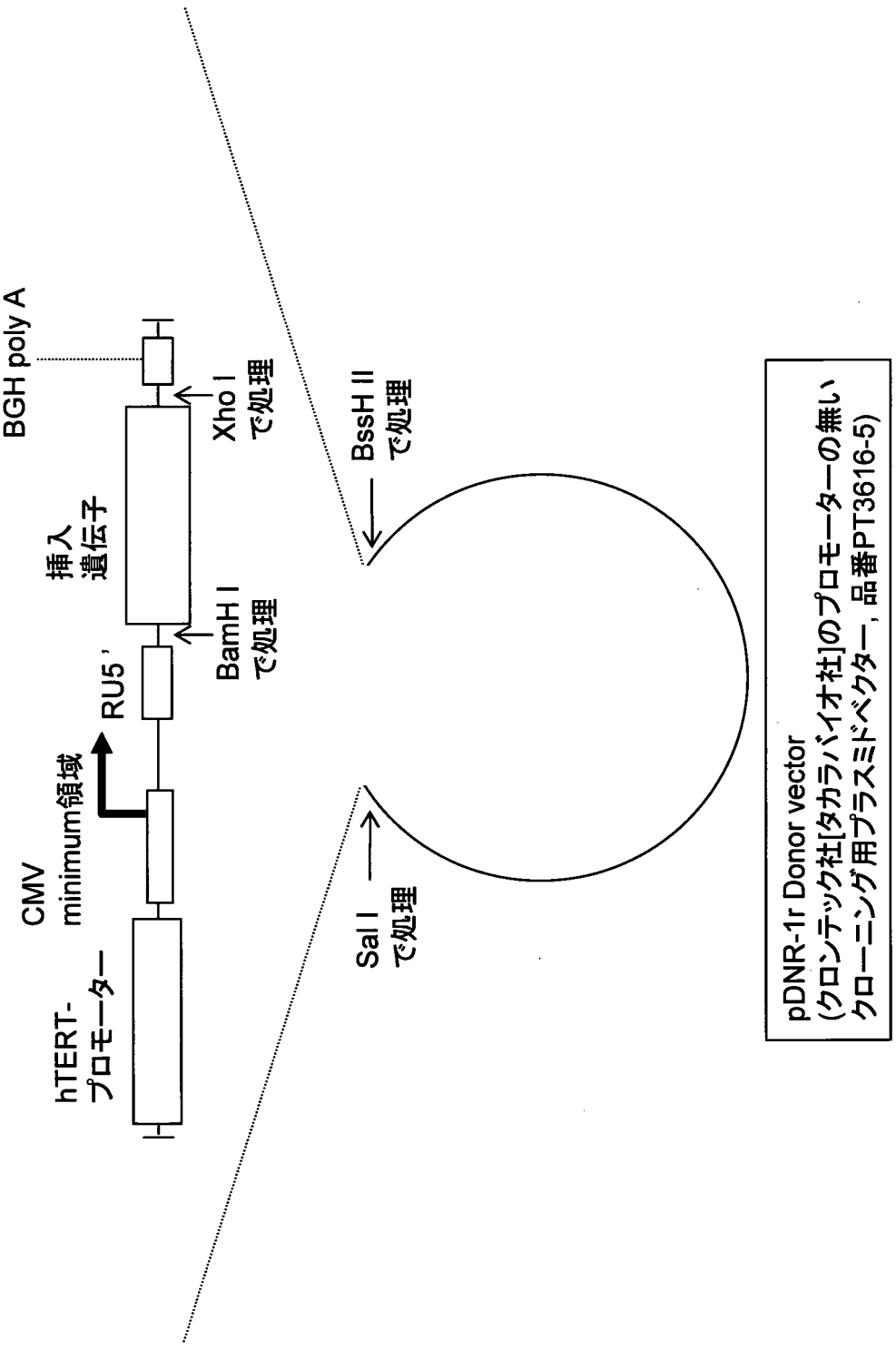


図５７

コンストラクト No.20

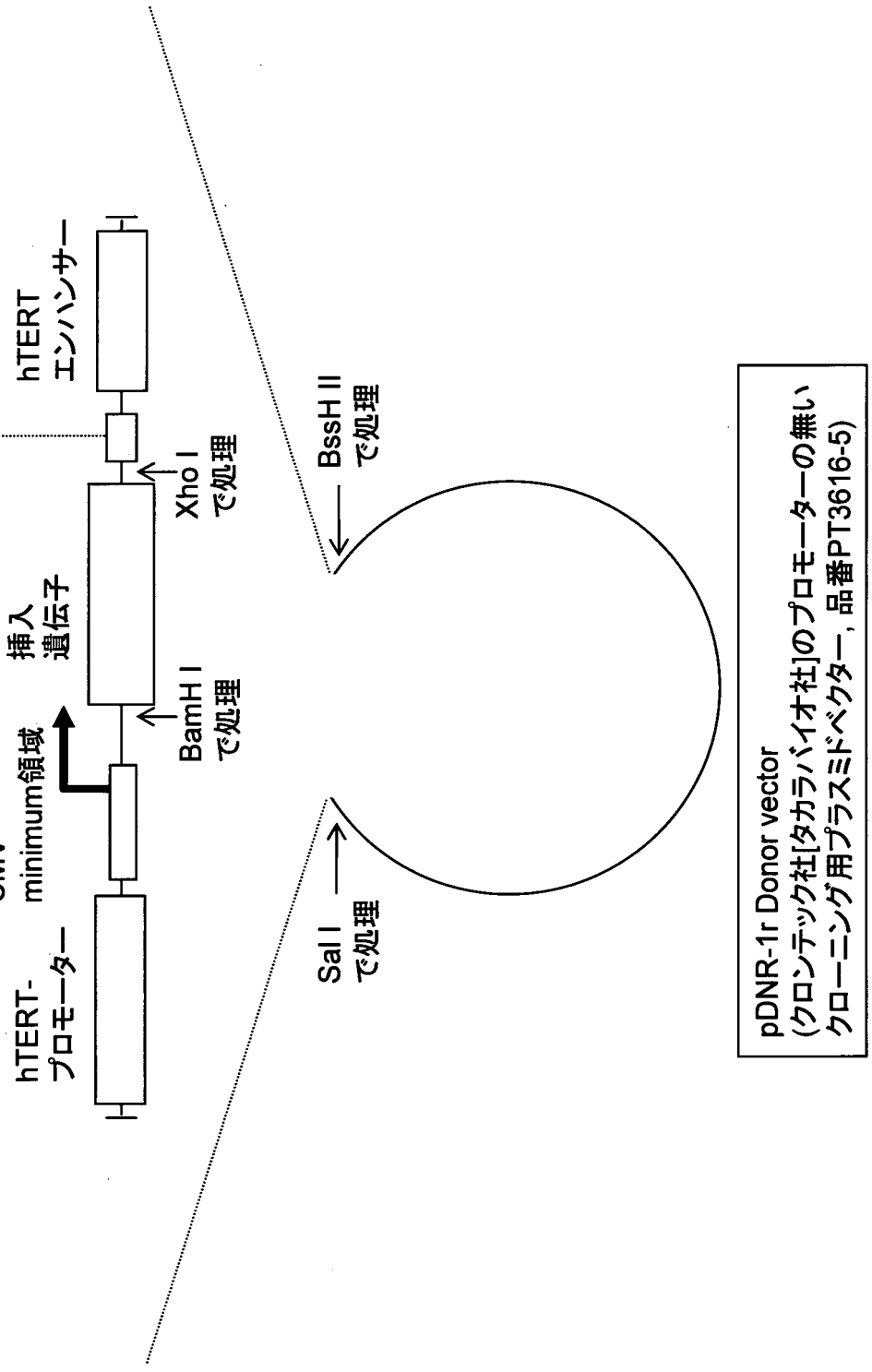


図５８

コンストラクト No.21

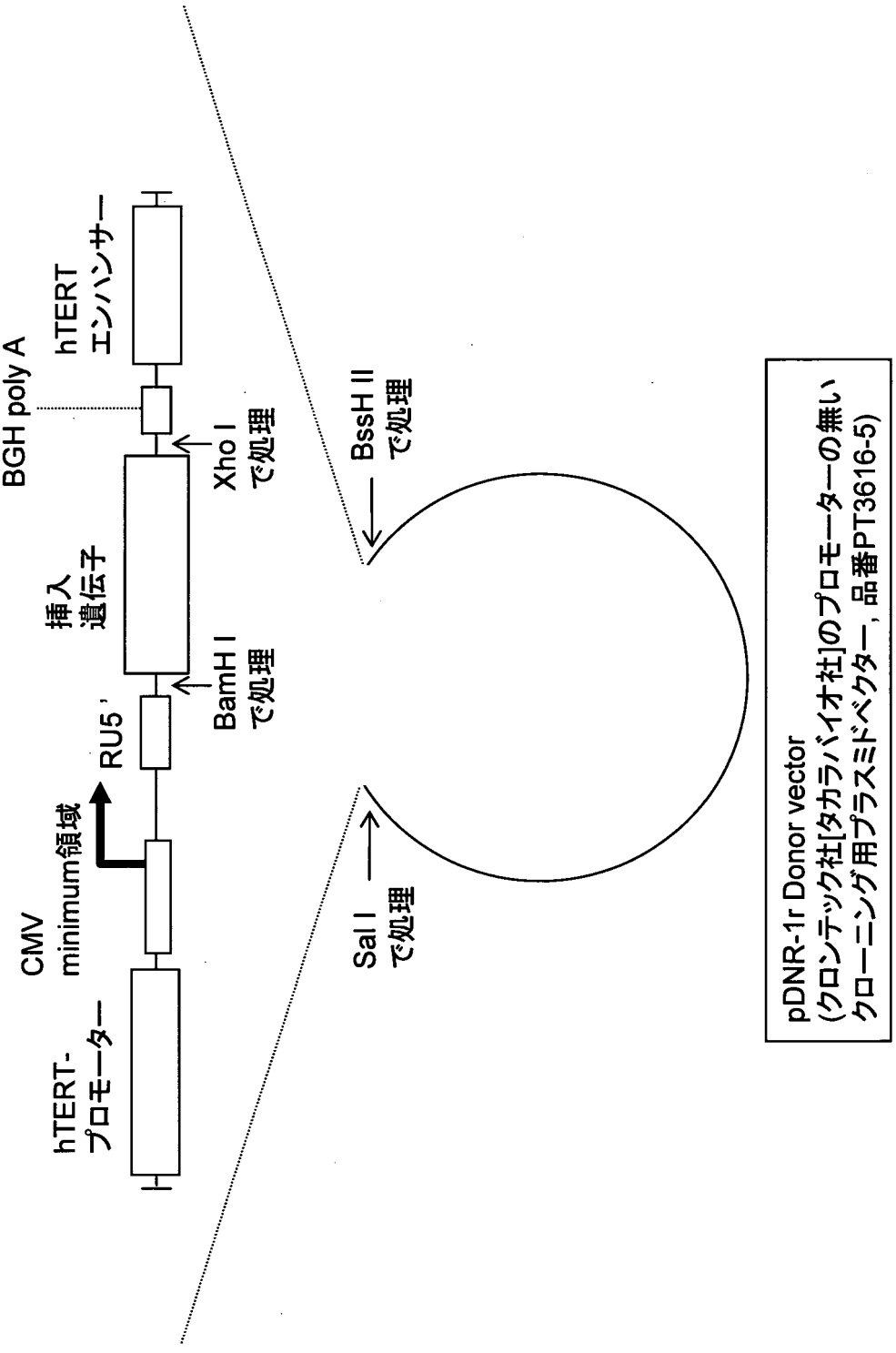


図59－1

#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
ttc gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag
tga CCGCTCGAGCGG GCTCTAGAG

図５９－２


GTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCC
CTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATG
AGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAG
GACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTAT
GG ATGCATCCAATGCATTGGATGCAT
cttcccacgtggcggagggactggggacccgggcacccgtcctgcccccttcaccttccagct
ccgcctcctccgcgcggaccccgccccgtcccgacccctcccgggtccccggcccagccccc
tccgggccctcccagcccctcccccttcctttccgcggccccgccctctcctcgcggcgcgag
tttcAGGCAGCGCTGCGTCCTGCTGCGCACGTGG TTGGCGCGC
(5)
(6)

図60

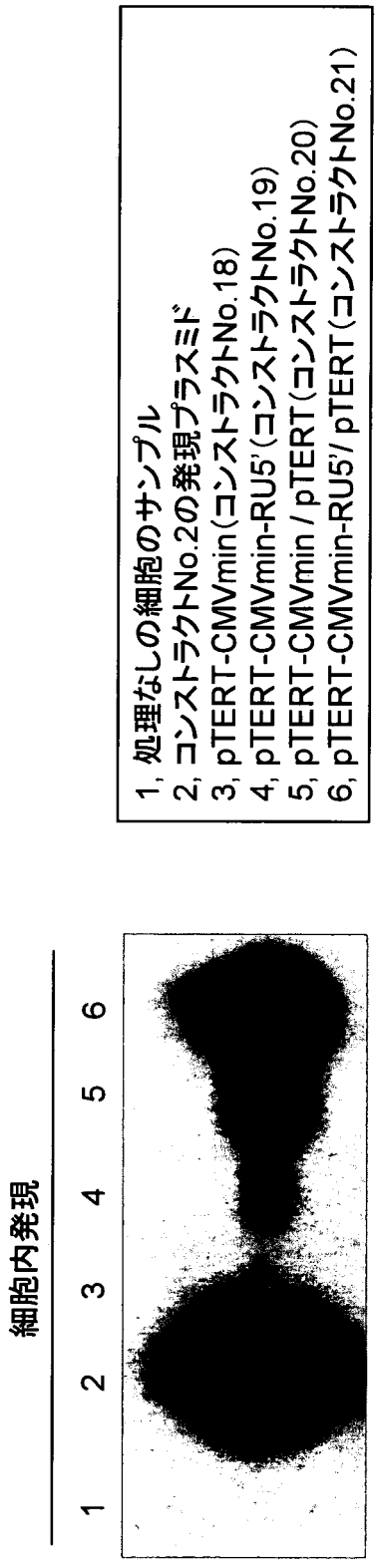

1, 処理なしの細胞のサンプル
2, コンストラクトNo.2の発現プラスミド
3, pTERT-CMVmin(コンストラクトNo.18)
4, pTERT-CMVmin-RU5'(コンストラクトNo.19)
5, pTERT-CMVmin / pTERT(コンストラクトNo.20)
6, pTERT-CMVmin-RU5'/ pTERT(コンストラクトNo.21)
細胞内発現
1
2
3
4
5
6

図６１

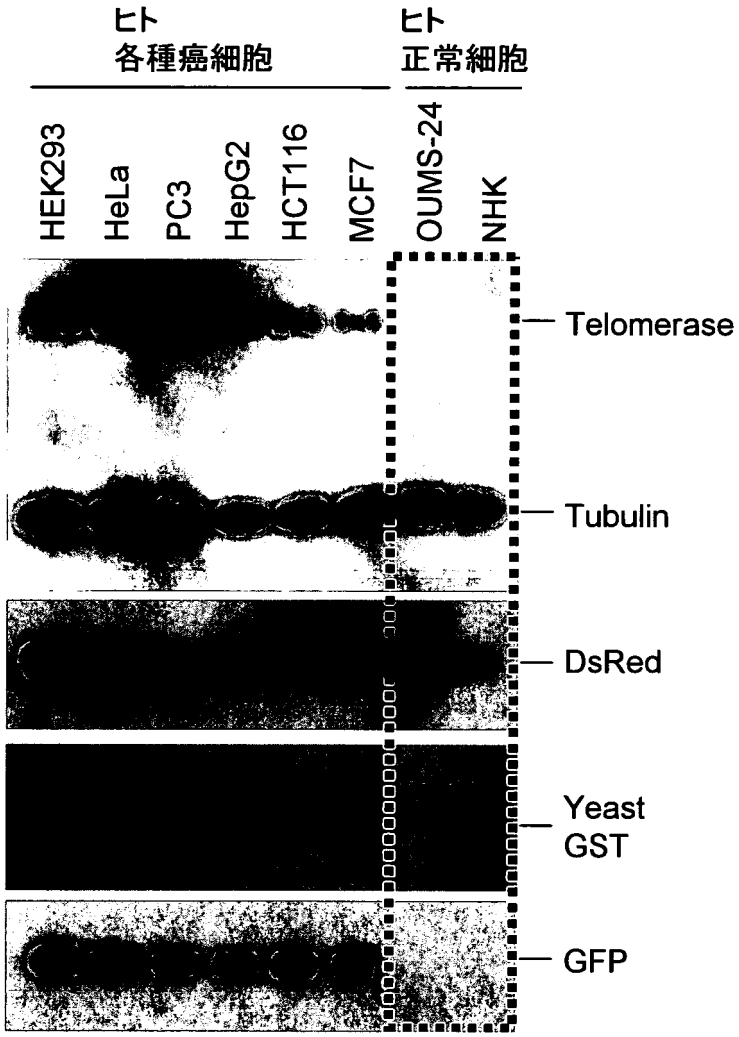
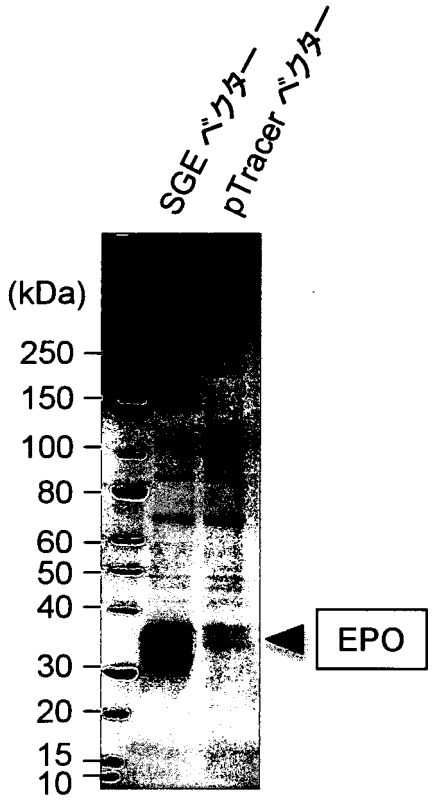
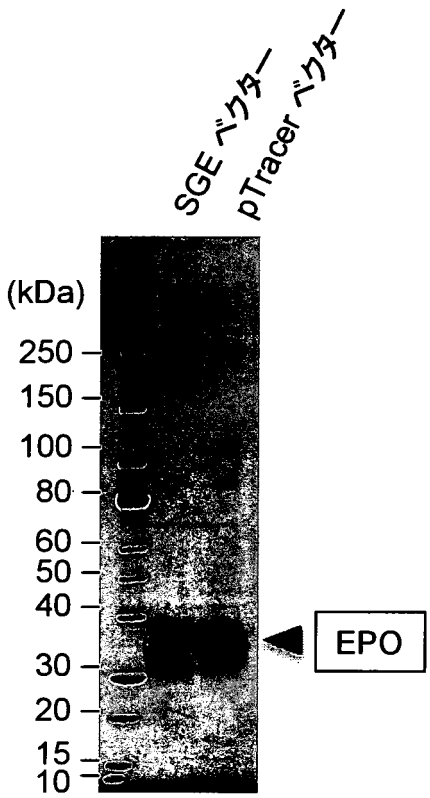

ヒト
各種癌細胞
ヒト
正常細胞
HEK293
HeLa
PC3
HepG2
HCT116
MCF7
OUMS-24
NHK
Telomerase
Tubulin
DsRed
Yeast
GST
GFP

図６２



CBB 染色

図６３



CBB 染色

図６４

A

25mLの培養上清をヒスチジン親和性カラムクロマトグラフィーにより精製後の タンパク質量

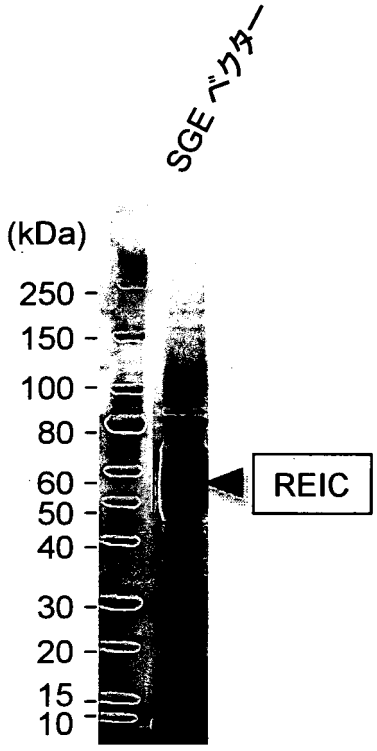
SGE ベクター	3.83mg
pTracer ベクター	0.49mg

B

1Lの培養上清に換算したタンパク質回収量

SGE ベクター	153.2mg
pTracer ベクター	19.6mg

図６５



CBB 染色

図６６

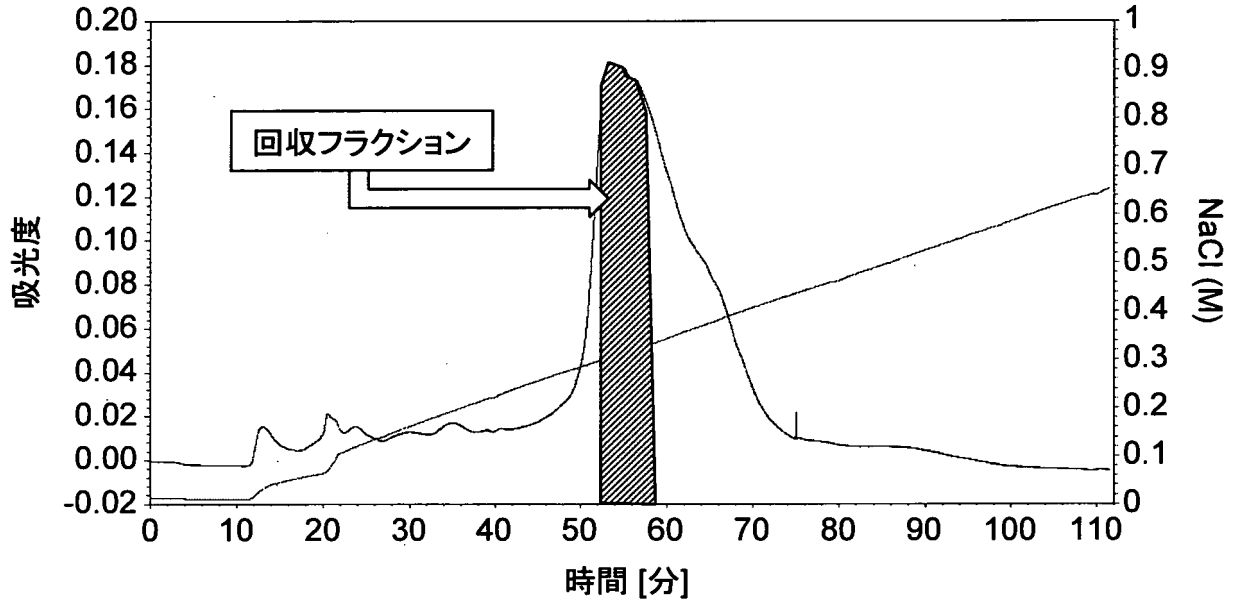

回収フラクション
吸光度
NaCl (M)
時間 [分]
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
80
90
100
110

図６７

REIC発現SGE ベクターをトランスフェクトした
培養上清を精製後の タンパク質量

520mLの培養上清から 精製後の タンパク質量	51mg
1Lの培養上清に換算した タンパク質回収量	98mg