FIG. 1.

pGN1

gagtgcaccatatgcggtgtgaaataccgcacagatgcgtaaggagaaaataccgcatcaggcgaaattgtaaacgttaatatttt
tgttaaaattcgcgttaaatatttgttaaatcagctcatttttaaccaataggccgaaatcggcaaaatcccttataaatcaaaagaat
agaccgagatagggttgagtgttgttccagtttggaacaagagtccactattaaagaacgtggactccaacgtcaaagggcgaa
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cggaaccctaaagggagcccccgatttagagcttgacggggaaagccggcgaacgtggcgagaaaggaagggaagaaag
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cgctacagggcgcgtccattcgccattcaggctgcgcaactgttgggaagggcgatcggtgcgggcctcttcgctattacgcca
gctggcgaaaggggatgtgctgcaaggcgattaagttgggtaacgccagggttttcccagtcacgacgttgtaaaacgacgg
ccagtgaattgtaatacgactcactatagggcgaattcgagctcggtacccggggatcctctagagtcgaaagcttctcgccctat
agtgagtcgtattacagcttgagtattctatagtgtcacctaaatagcttggcgtaatcatggtcatagctgtttcctgtgtgaaattgtt
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atgagtaaacttggtctgacagttaccaatgcttaatcagtgaggcacctatctcagcgatctgtctatttcgttcatccatagttgcct
gactccccgtcgtgtagataactacgatacgggagggcttaccatctggccccagtgctgcaatgataccgcgagacccacgct

FIG. 1. (CONTINUED)

caccggctccagatttatcagcaataaaccagccagccggaagggccgagcgcagaagtggtcctgcaactttatccgcctcc
atccagtctattaattgttgccgggaagctagagtaagtagttcgccagttaatagtttgcgcaacgttgttggcattgctacaggca
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ggcgaccgagttgctcttgcccggcgtcaatacgggataatagtgtatgacatagcagaactttaaaagtgctcatcattggaaaa
cgttcttcggggcgaaaactctcaaggatcttaccgctgttgagatccagttcgatgtaacccactcgtgcacccaactgatcttca
gcatcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgccgcaaaaaagggaataagggcgacacg
gaaatgttgaatactcatactcttcctttttcaatattattgaagcatttatcagggttattgtctcatgagcggatacatatttgaatgtat
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gtcggggctggcttaactatgcggcatcagagcagattgtactga

FIG. 2.

PGN100

ctagcatgaacacgattaacatcgctaagaacgacttctctgacatcgaactggctgctatcccgttcaacactctggctgaccatt
acggtgagcgtttagctcgcgaacagttggcccttgagcatgagtcttacgagatgggtgaagcacgcttccgcaagatgtttga
gcgtcaacttaaagctggtgaggttgcggataacgctgccgccaagcctctcatcactaccctactccctaagatgattgcacgc
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FIG. 2. (CONTINUED 1)

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FIG. 2. (CONTINUED 2)

ctagaaggtcctagatgcattcgtttgaaaatactcccggtgggtgcaaagagacgcagacggaaaatgtatctgggtctctttatt
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gtataaataccgttgaaaataaataccgaacaacatttgctctaattgtgaaattagaaatcttcaaactataatcatctcactggatc
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FIG. 3.

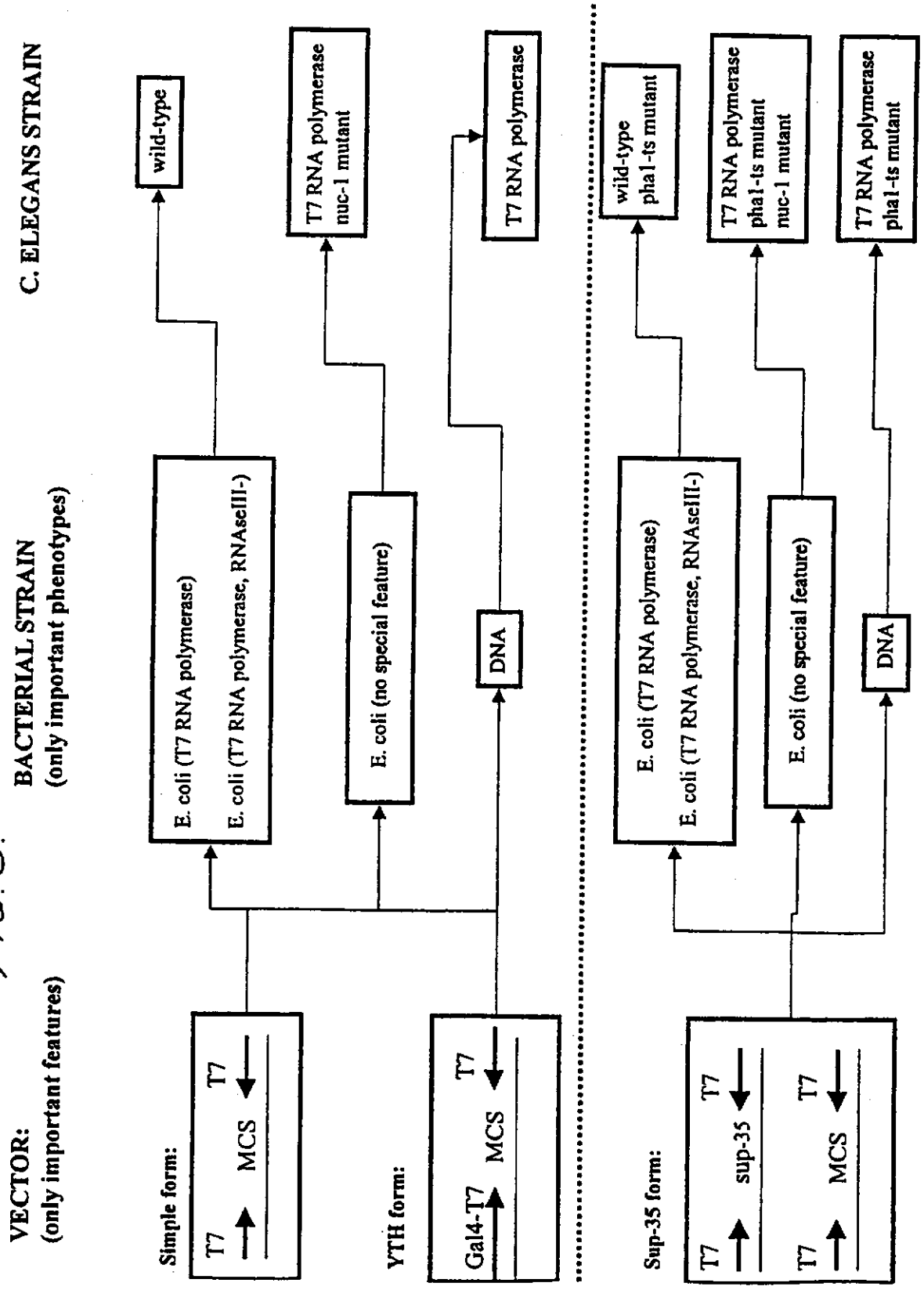
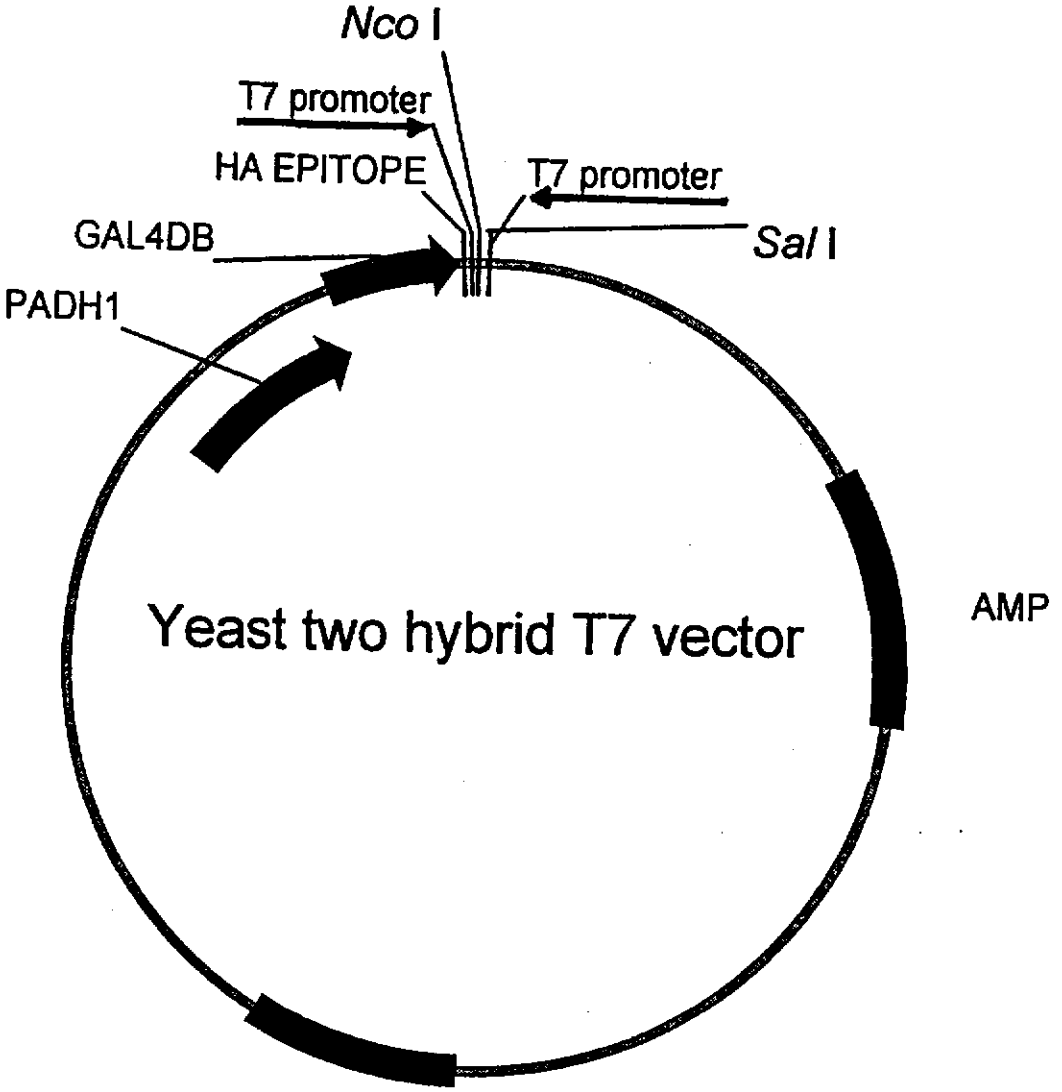

VECTOR:
(only important features)
BACTERIAL STRAIN
(only important phenotypes)
C. ELEGANS STRAIN
Simple form:
T7
MCS
T7
E. coli (T7 RNA polymerase)
E. coli (T7 RNA polymerase, RNAseIII-)
wild-type
E. coli (no special feature)
T7 RNA polymerase nuc-1 mutant
YTH form:
Gal4-T7
MCS
T7
DNA
T7 RNA polymerase
Sup-35 form:
T7
sup-35
T7
T7
MCS
T7
E. coli (T7 RNA polymerase)
E. coli (T7 RNA polymerase, RNAseIII-)
wild-type pha1-ts mutant
E. coli (no special feature)
T7 RNA polymerase pha1-ts mutant nuc-1 mutant
DNA
T7 RNA polymerase pha1-ts mutant

FIG. 4.


Nco I
T7 promoter
HA EPITOPE
T7 promoter
GAL4DB
Sal I
PADH1
Yeast two hybrid T7 vector
AMP
TRP1

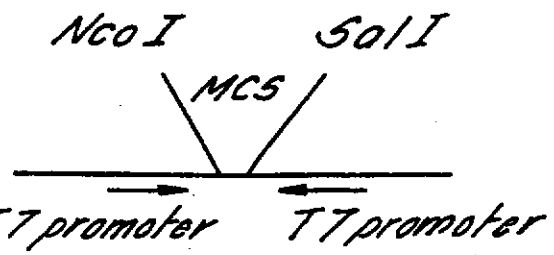
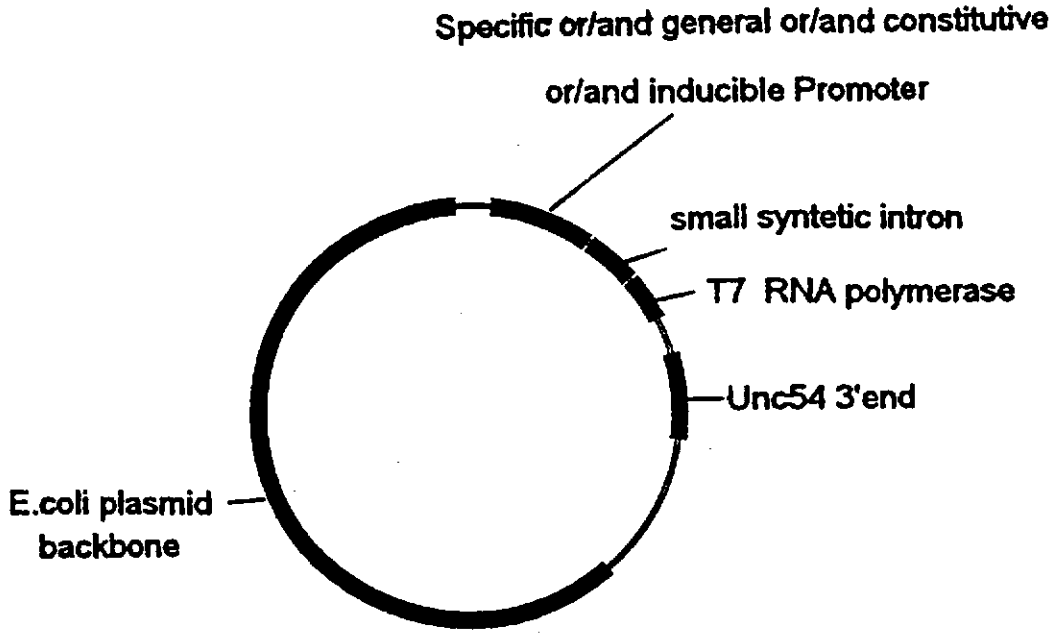

Nco I
Sal I
MCS
T7 promoter
T7 promoter

FIG. 5.

General description of the C.elegans T7 RNA polymerase expression vector with 4 examples



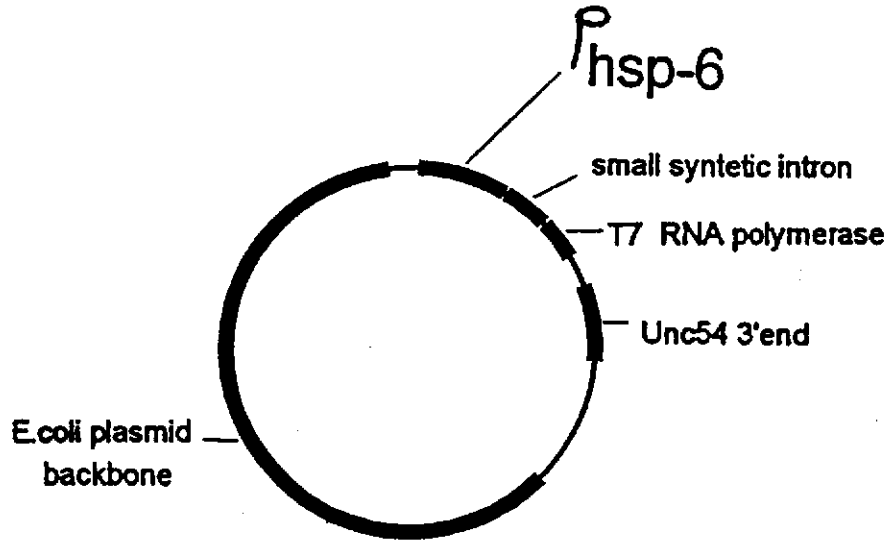


FIG. 5. (CONTINUED)

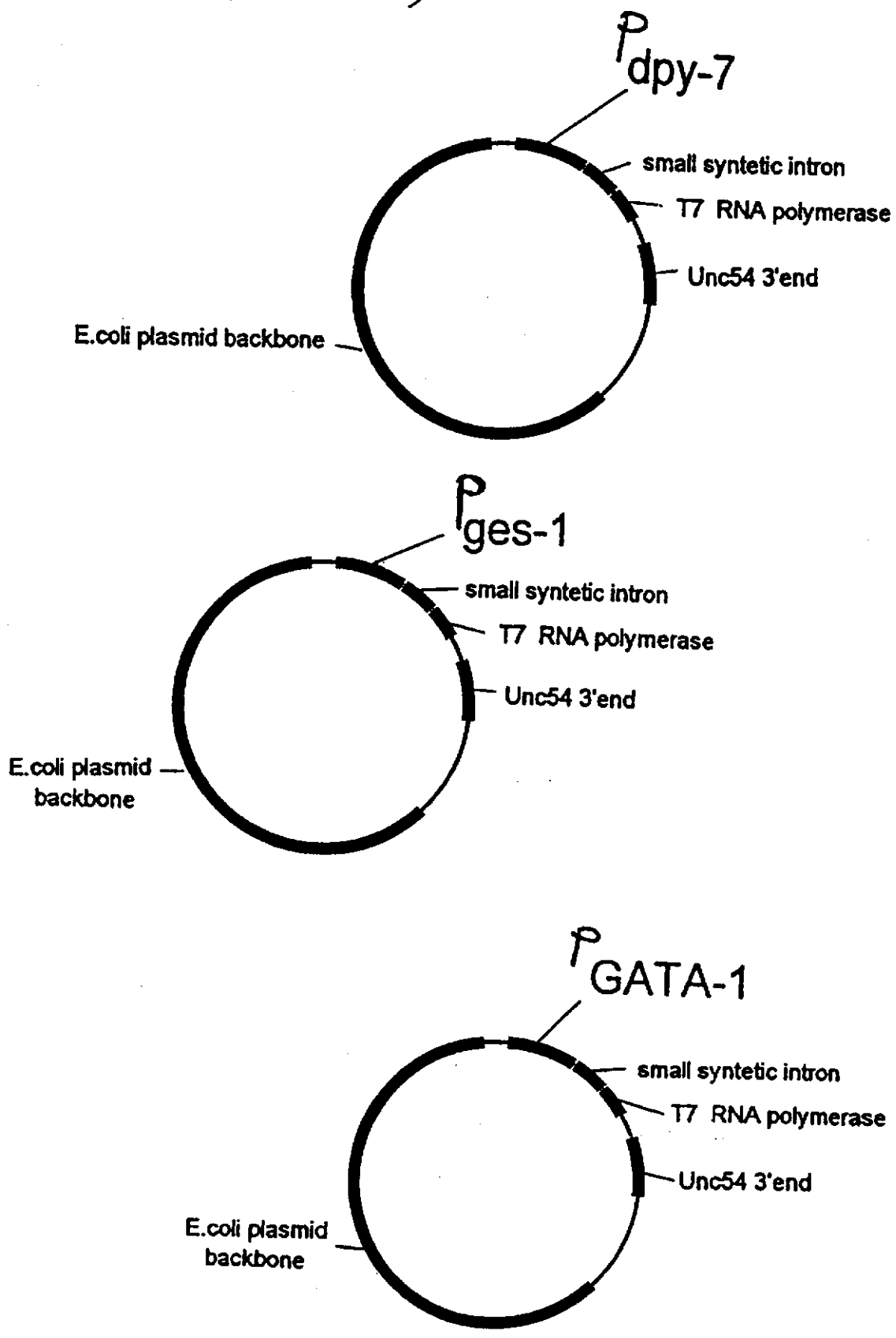


FIG. 6.

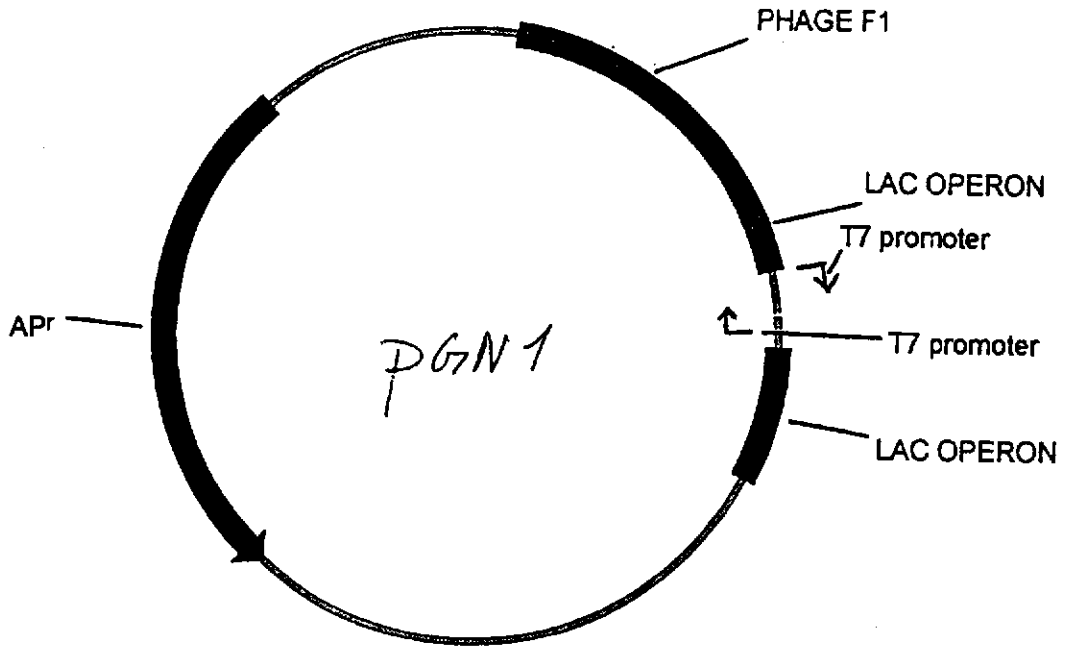
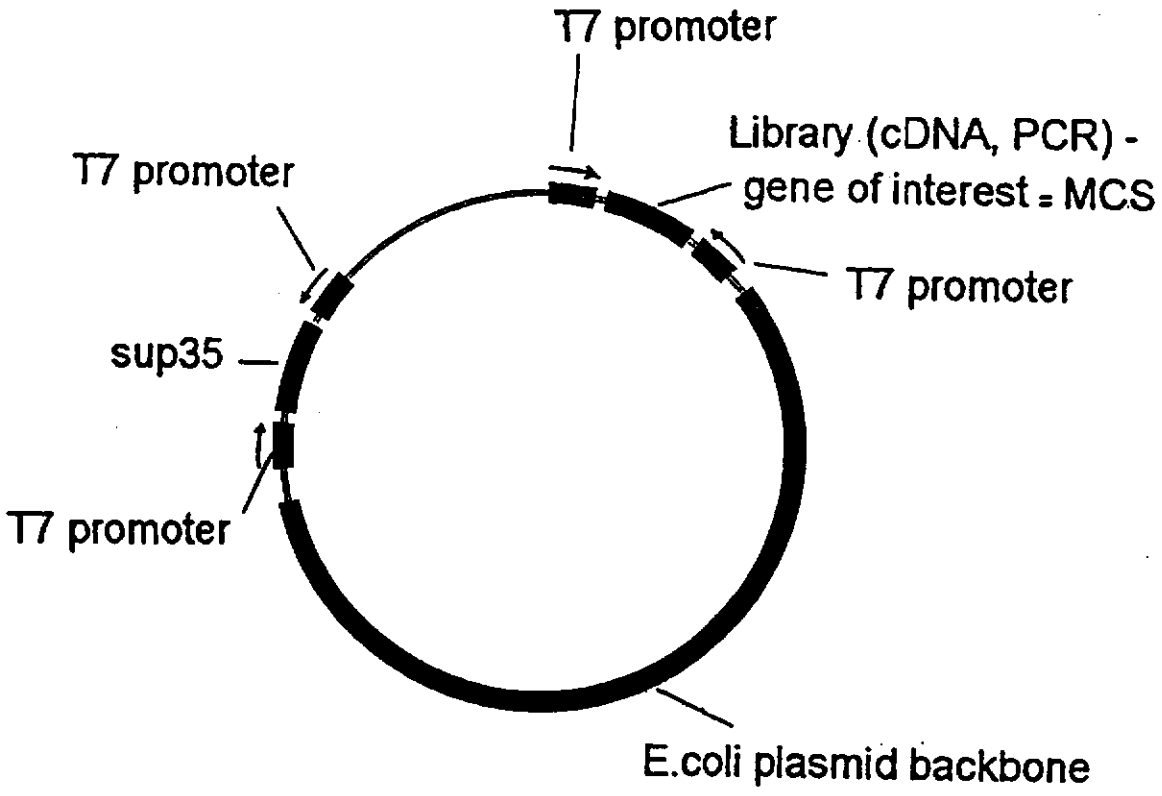

PHAGE F1
LAC OPERON
T7 promoter
T7 promoter
LAC OPERON
APr
PGN1

FIG. 7



enhanced vector for RNAi, producing sup35 dsRNA and dsRNA of the library, gene of interest or PCR product.

FIG. 8.

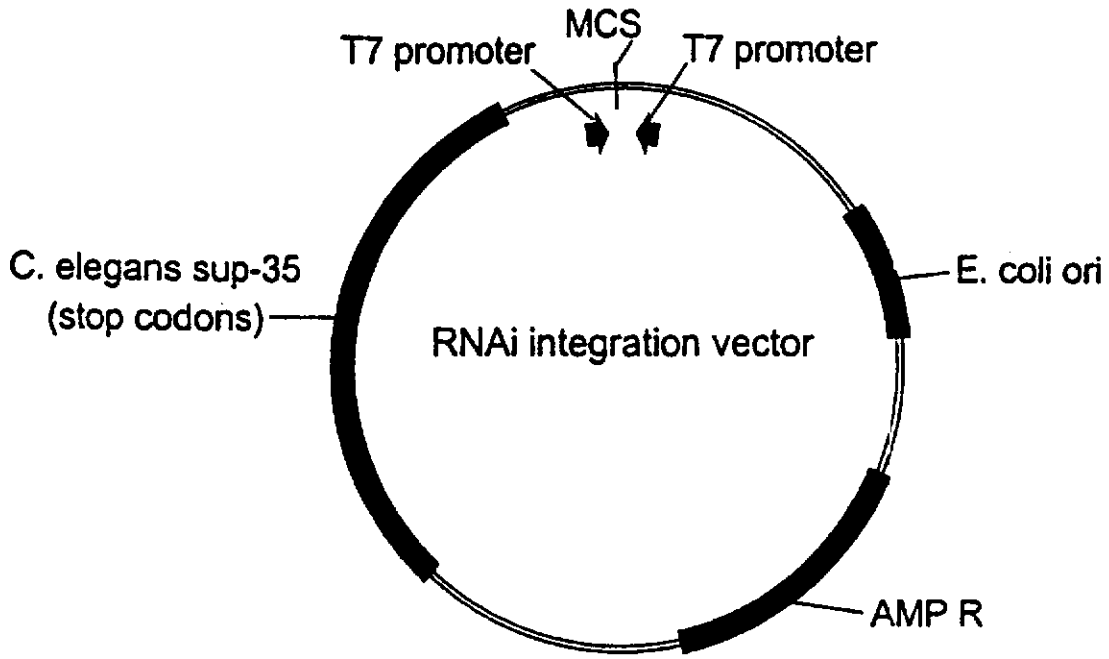

MCS
T7 promoter
T7 promoter
C. elegans sup-35
(stop codons)
RNAi integration vector
E. coli ori
AMP R

FIG. 9.

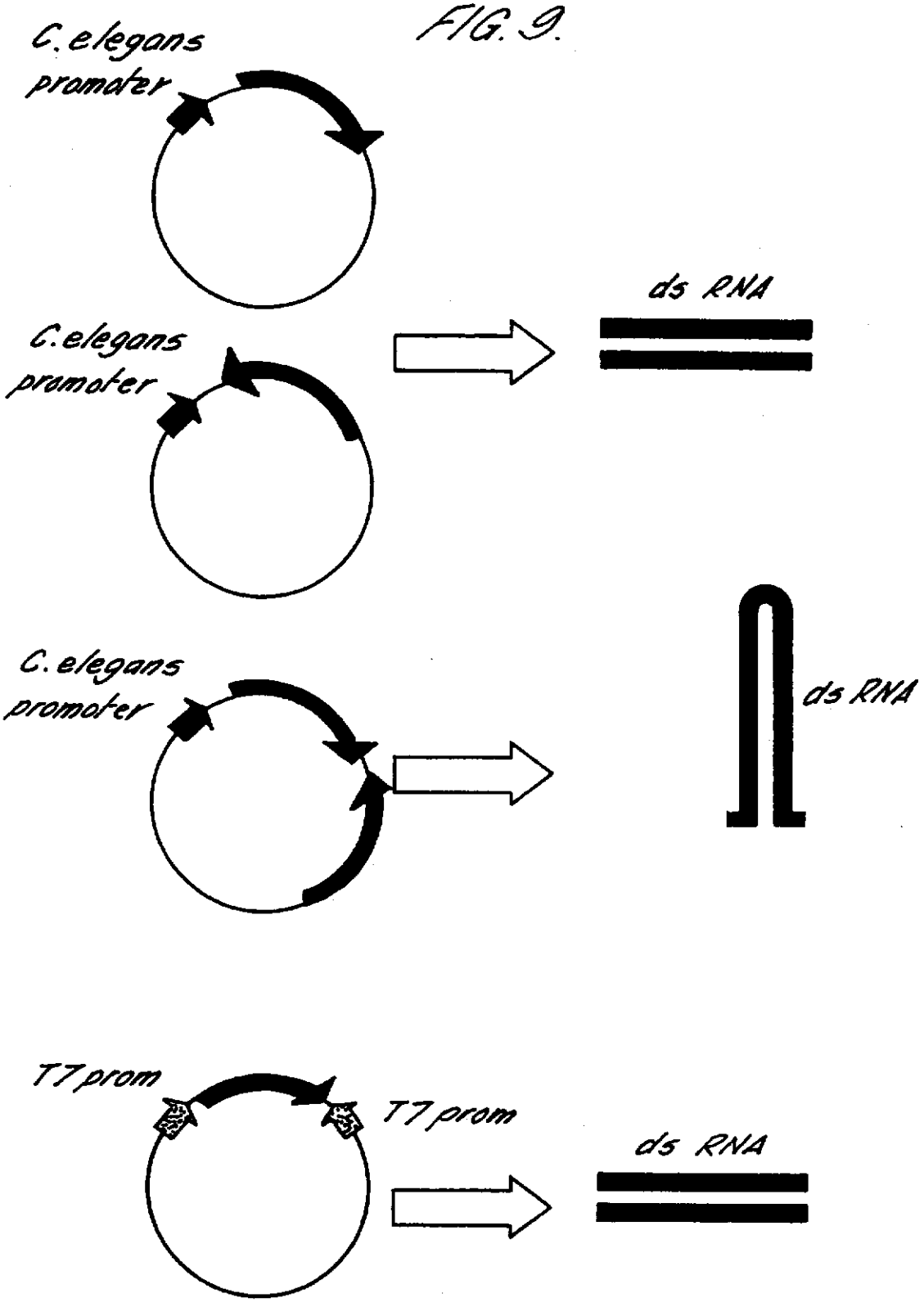

C. elegans promoter
ds RNA
C. elegans promoter
C. elegans promoter
ds RNA
T7 prom
T7 prom
ds RNA

FIG. 10.

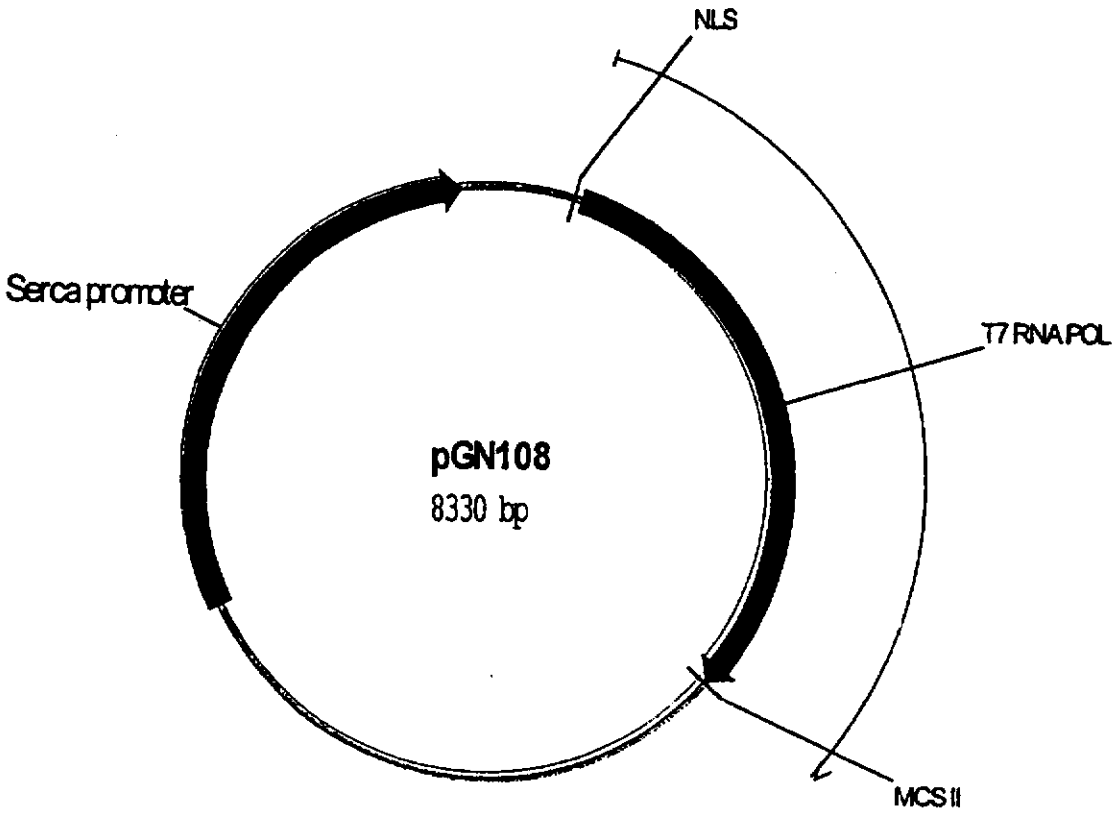


FIG. 11.

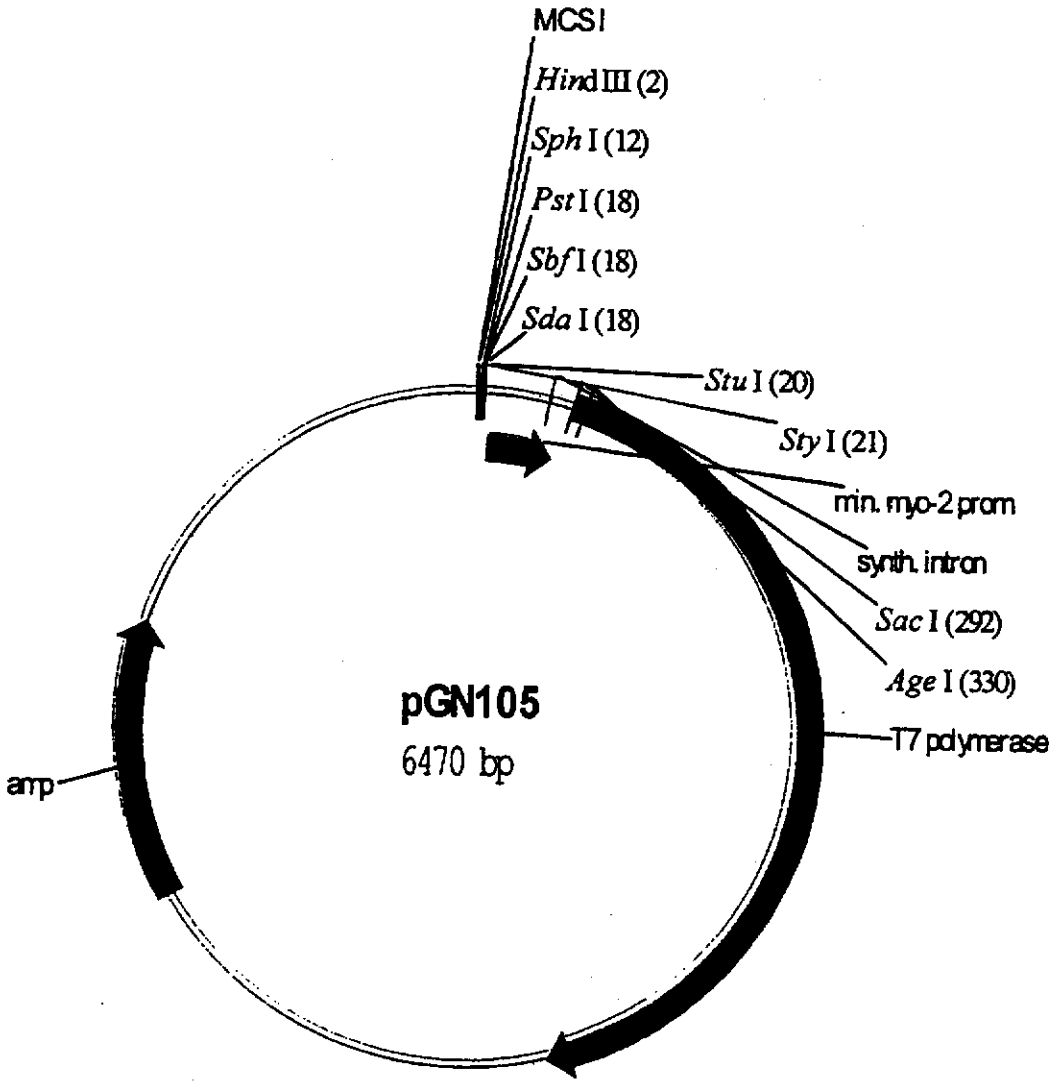

MCS I
Hind III (2)
Sph I (12)
Pst I (18)
Sbf I (18)
Sda I (18)
Stu I (20)
Sty I (21)
min. myo-2 prom
synth. intron
Sac I (292)
Age I (330)
T7 polymerase
amp
pGN105
6470 bp

FIG. 12.

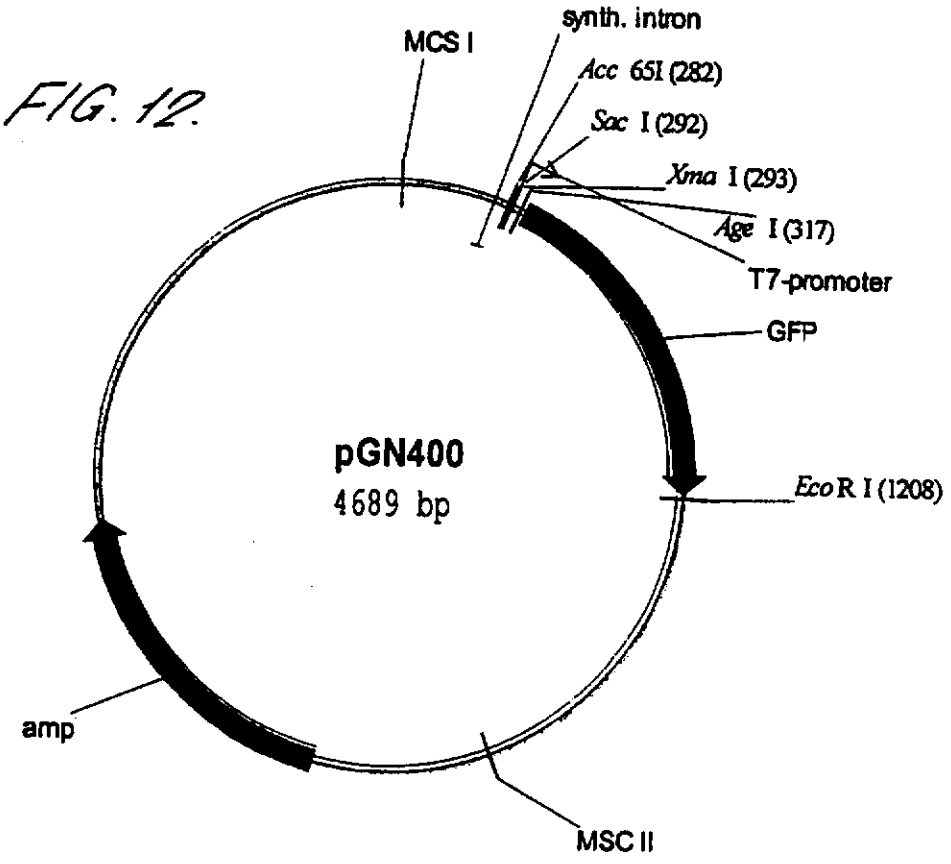

MCS I
synth. intron
Acc 65I (282)
Sac I (292)
Xma I (293)
Age I (317)
T7-promoter
GFP
pGN400
4689 bp
EcoR I (1208)
amp
MSC II

FIG. 13.

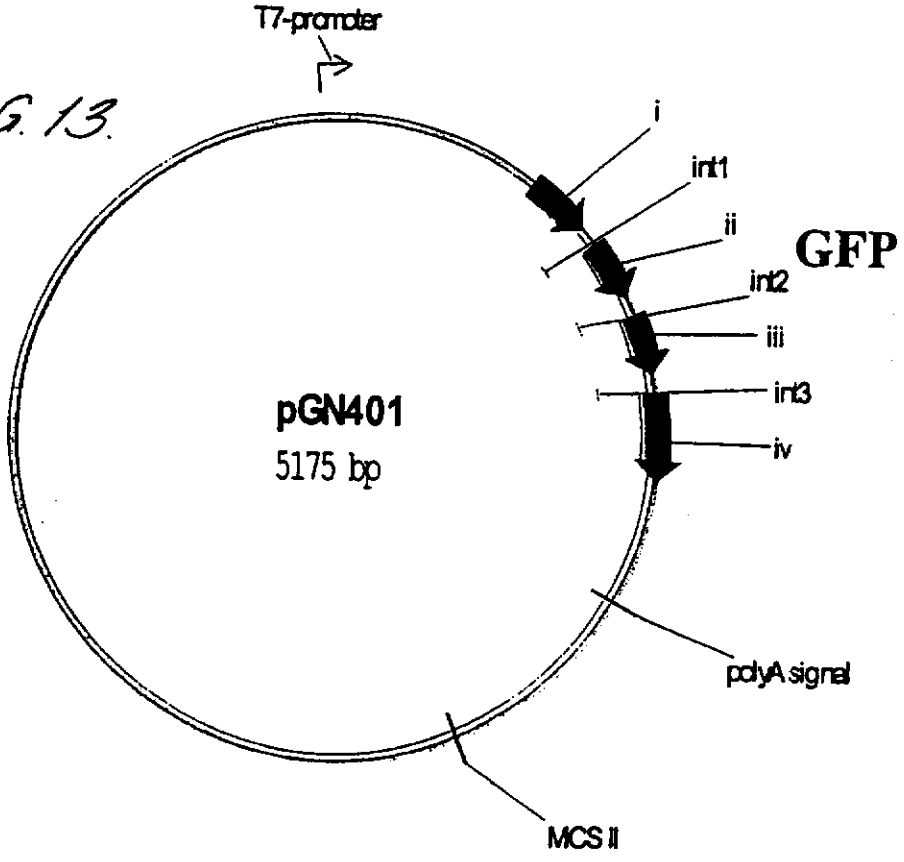

T7-promoter
i
int1
ii
int2
iii
int3
iv
GFP
pGN401
5175 bp
polyA signal
MCS II

FIG. 14.

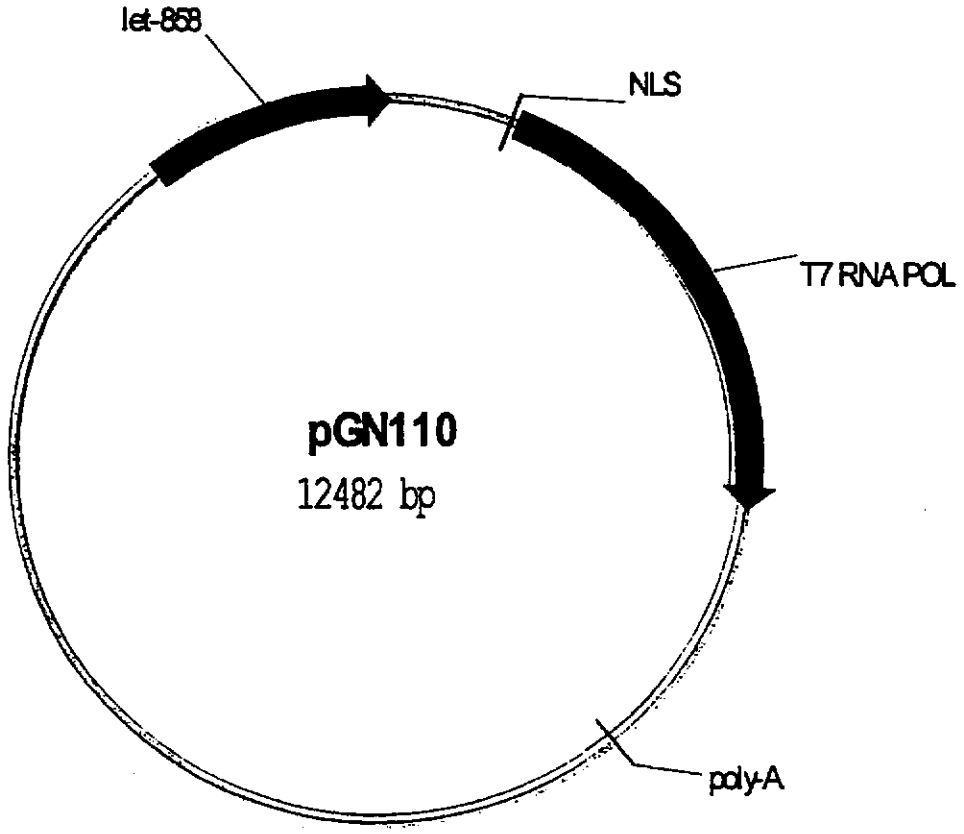


FIG. 15.

pAS2* with Forward and Reverse T7/T3/Sp6

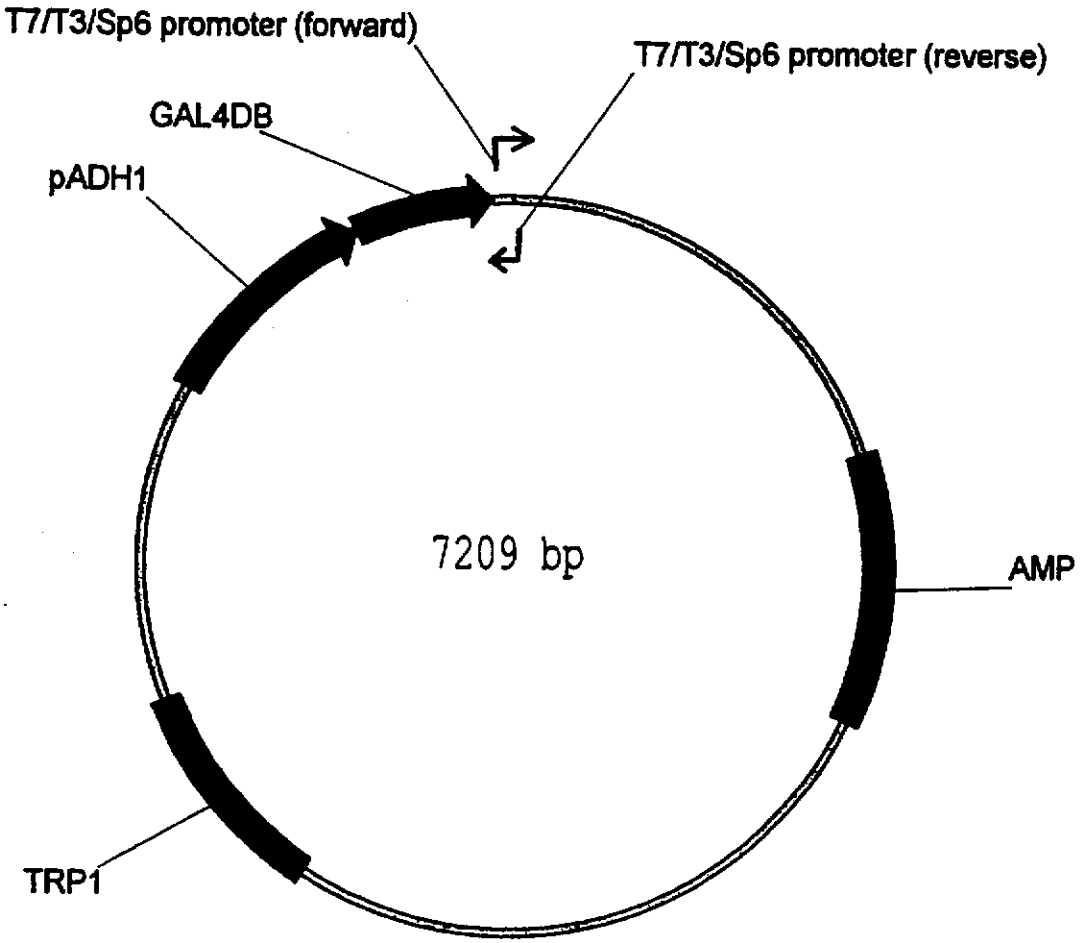


FIG. 16.

pGAD424 with Forward and Reverse T7/T3/Sp6

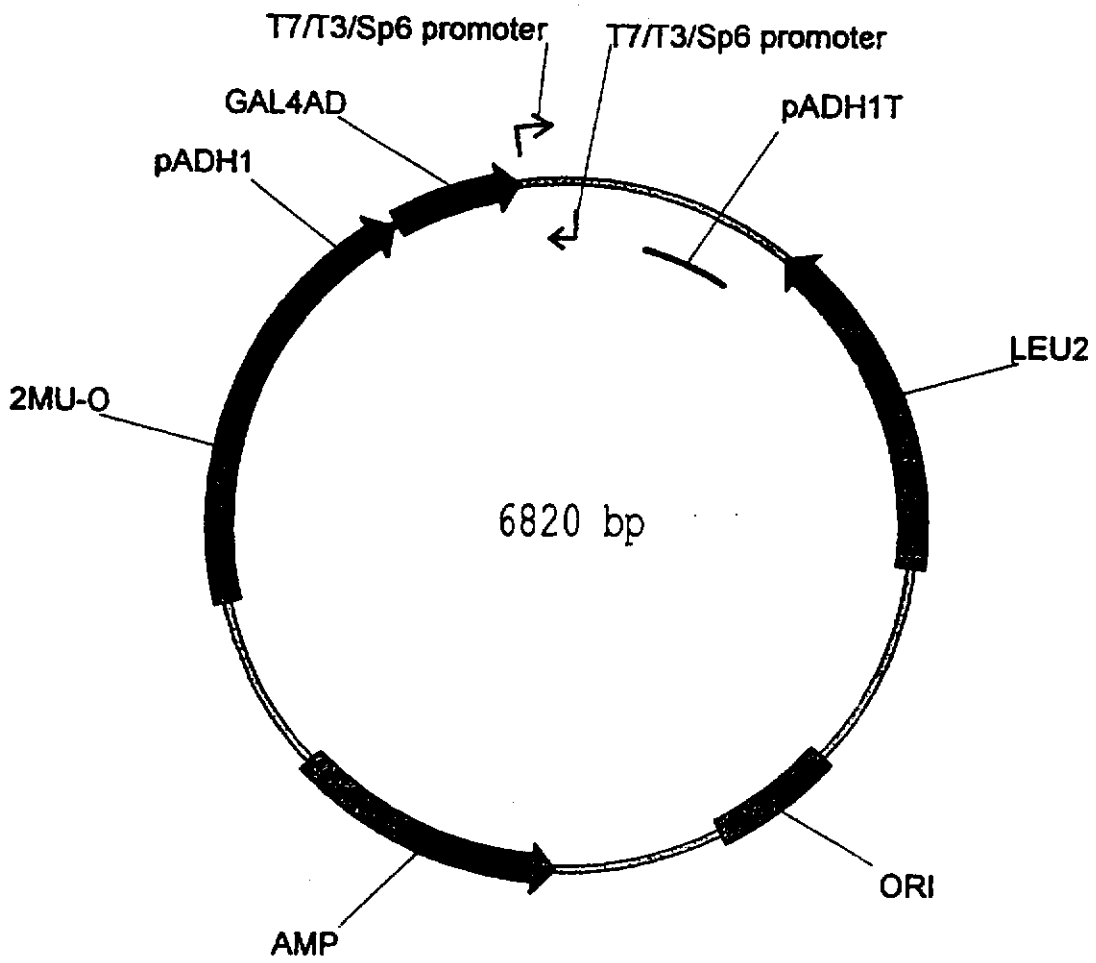


FIG. 17.

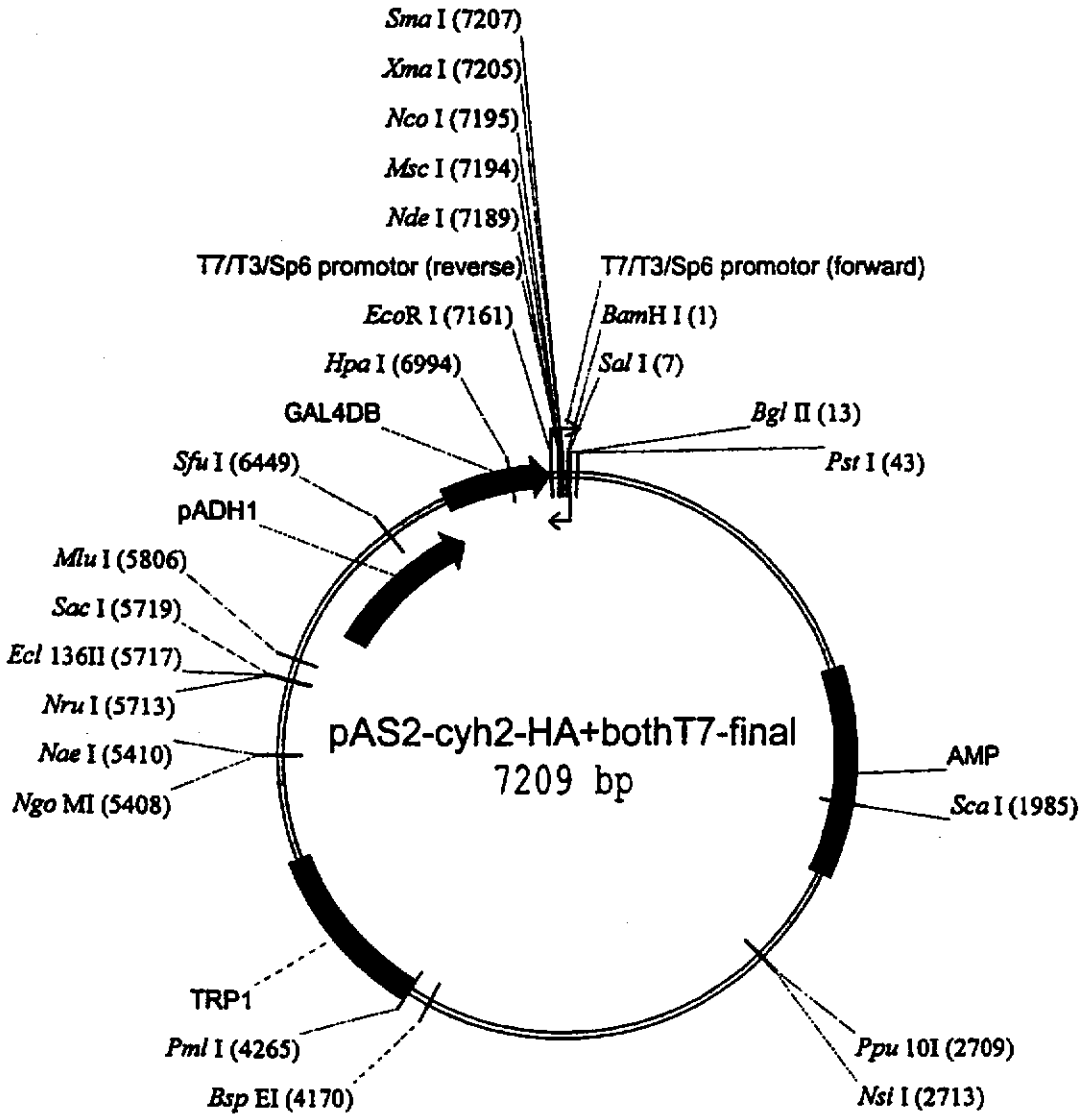

Sma I (7207)
Xma I (7205)
Nco I (7195)
Msc I (7194)
Nde I (7189)
T7/T3/Sp6 promotor (reverse)
T7/T3/Sp6 promotor (forward)
EcoR I (7161)
BamH I (1)
Hpa I (6994)
Sal I (7)
GAL4DB
Bgl II (13)
Sfu I (6449)
Pst I (43)
pADH1
Mlu I (5806)
Sac I (5719)
Ecl 136II (5717)
Nru I (5713)
Nae I (5410)
Ngo MI (5408)
pAS2-cyh2-HA+bothT7-final
7209 bp
AMP
Sca I (1985)
TRP1
Pml I (4265)
Bsp EI (4170)
Ppu 10I (2709)
Nsi I (2713)

FIG. 18.

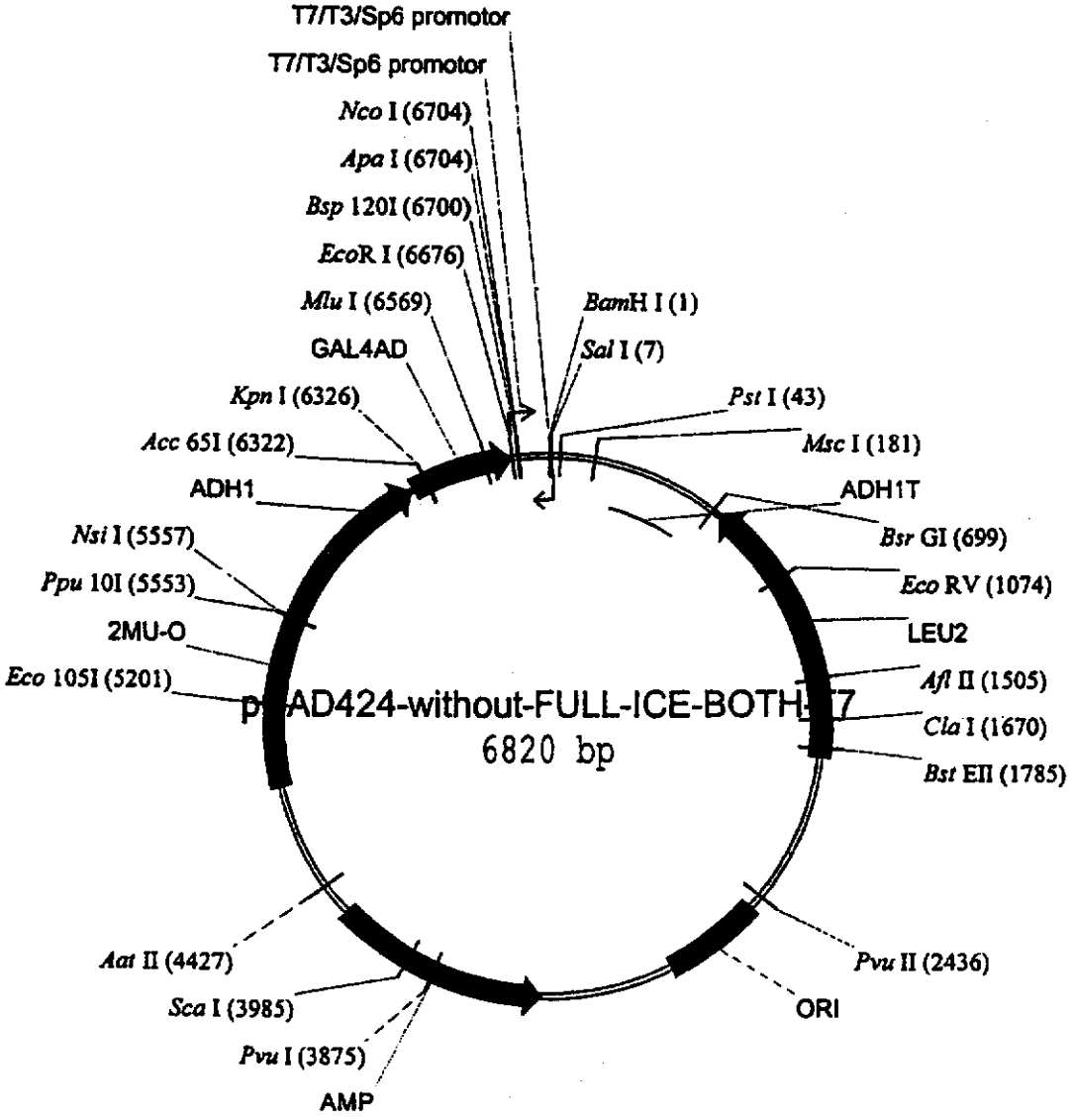
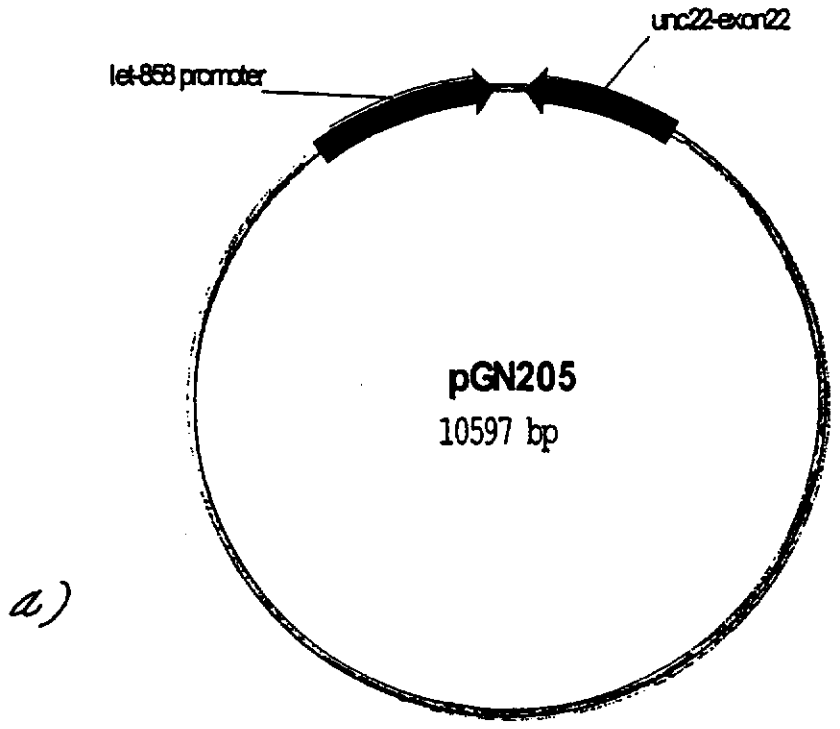
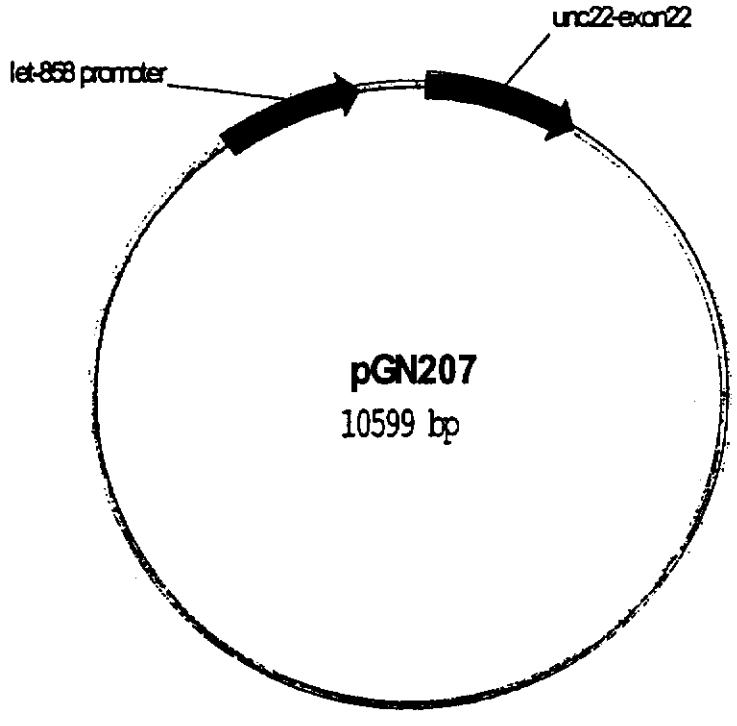

T7/T3/Sp6 promotor
T7/T3/Sp6 promotor
Nco I (6704)
Apa I (6704)
Bsp 120I (6700)
EcoR I (6676)
Mlu I (6569)
GAL4AD
Kpn I (6326)
Acc 65I (6322)
ADH1
Nsi I (5557)
Ppu 10I (5553)
2MU-O
Eco 105I (5201)
BamH I (1)
Sal I (7)
Pst I (43)
Msc I (181)
ADH1T
Bsr GI (699)
Eco RV (1074)
LEU2
Afl II (1505)
Cla I (1670)
Bst EII (1785)
6820 bp
Aat II (4427)
Sca I (3985)
Pvu I (3875)
AMP
Pvu II (2436)
ORI

FIG. 19


unc22-exon22
let-858 promoter
pGN205
10597 bp

a)


unc22-exon22
let-858 promoter
pGN207
10599 bp

b)