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(54) **PHAGEMID VECTORS**

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(21) Appl. No.: **11/986,963**

(22) Filed: **Nov. 26, 2007**

Related U.S. Patent Documents

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Filed: **Apr. 26, 2002**

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(51) **Int. Cl.**

C12N 15/63	(2006.01)
C12N 15/70	(2006.01)
C12N 15/73	(2006.01)
C12N 15/64	(2006.01)
C12N 15/65	(2006.01)
C07H 21/04	(2006.01)

(52) **U.S. Cl.** **435/320.1**; 536/23.1; 536/23.4; 536/23.7; 536/24.1; 536/23.2; 435/69.1; 435/5

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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Minute of the Oral Proceedings in EP Patent No. B–1390489 (App. No. 02 734 056.1) dated Dec. 17, 2008.
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Primary Examiner—Jennifer Dunston
(74) *Attorney, Agent, or Firm*—Ropes & Gray LLP

(57) **ABSTRACT**

Phagemid vectors containing a sequence of features between a Col E1 origin and an f1 origin are useful for display of polypeptides or proteins, including antibody libraries.

3 Claims, 46 Drawing Sheets

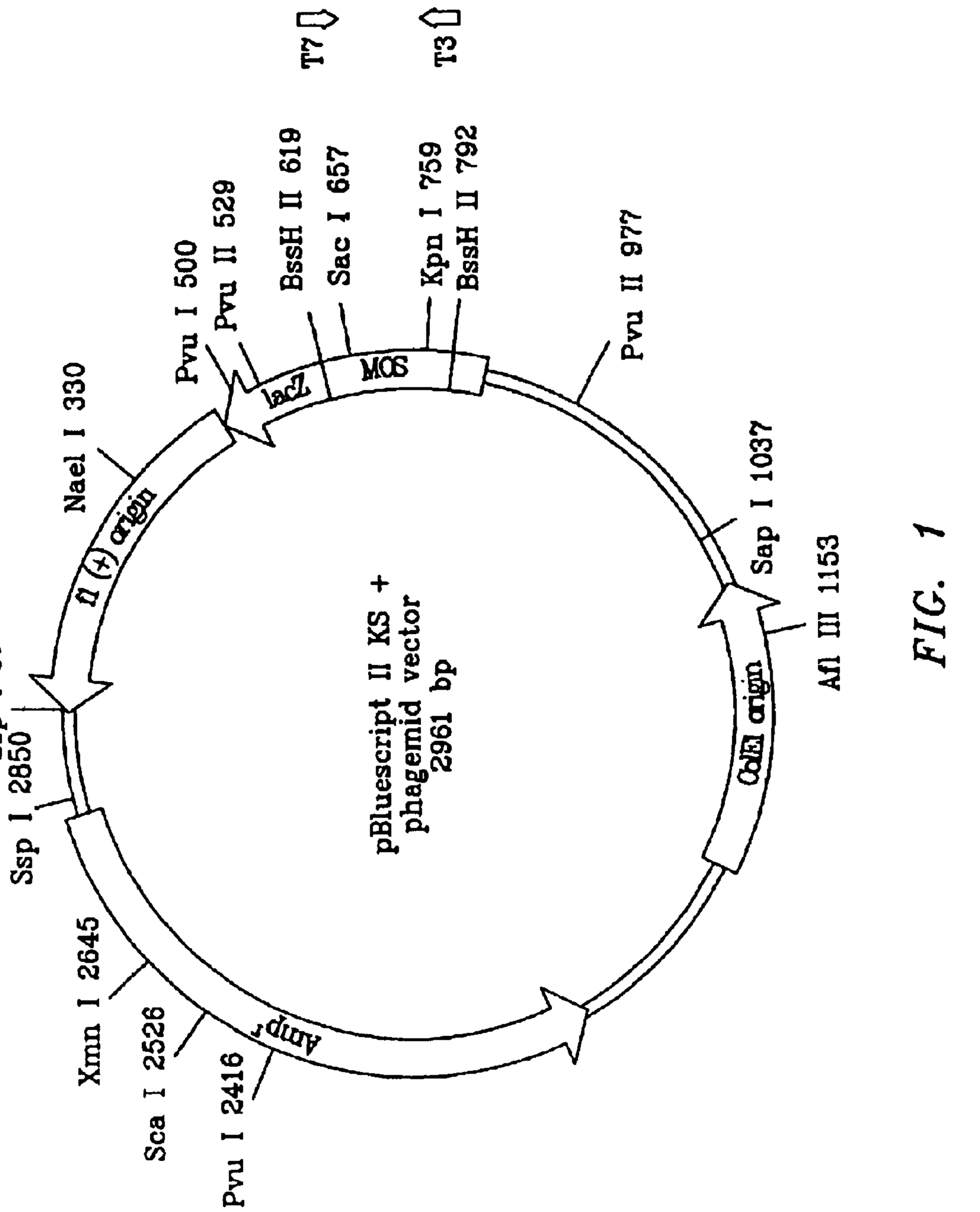

T7
T3
BssH II 619
Sac I 657
Kpn I 759
BssH II 792
Pvu I 500
Pvu II 529
Pvu II 977
NaeI I 330
lacZ
MCS
f1 (+) origin
pBluescript II KS +
phagemid vector
2961 bp
Sap I 1037
Afl III 1153
ColE1 origin
Ssp I 19
Ssp I 2850
Xmn I 2645
Sca I 2526
Pvu I 2416
Amp^r

FIG. 1

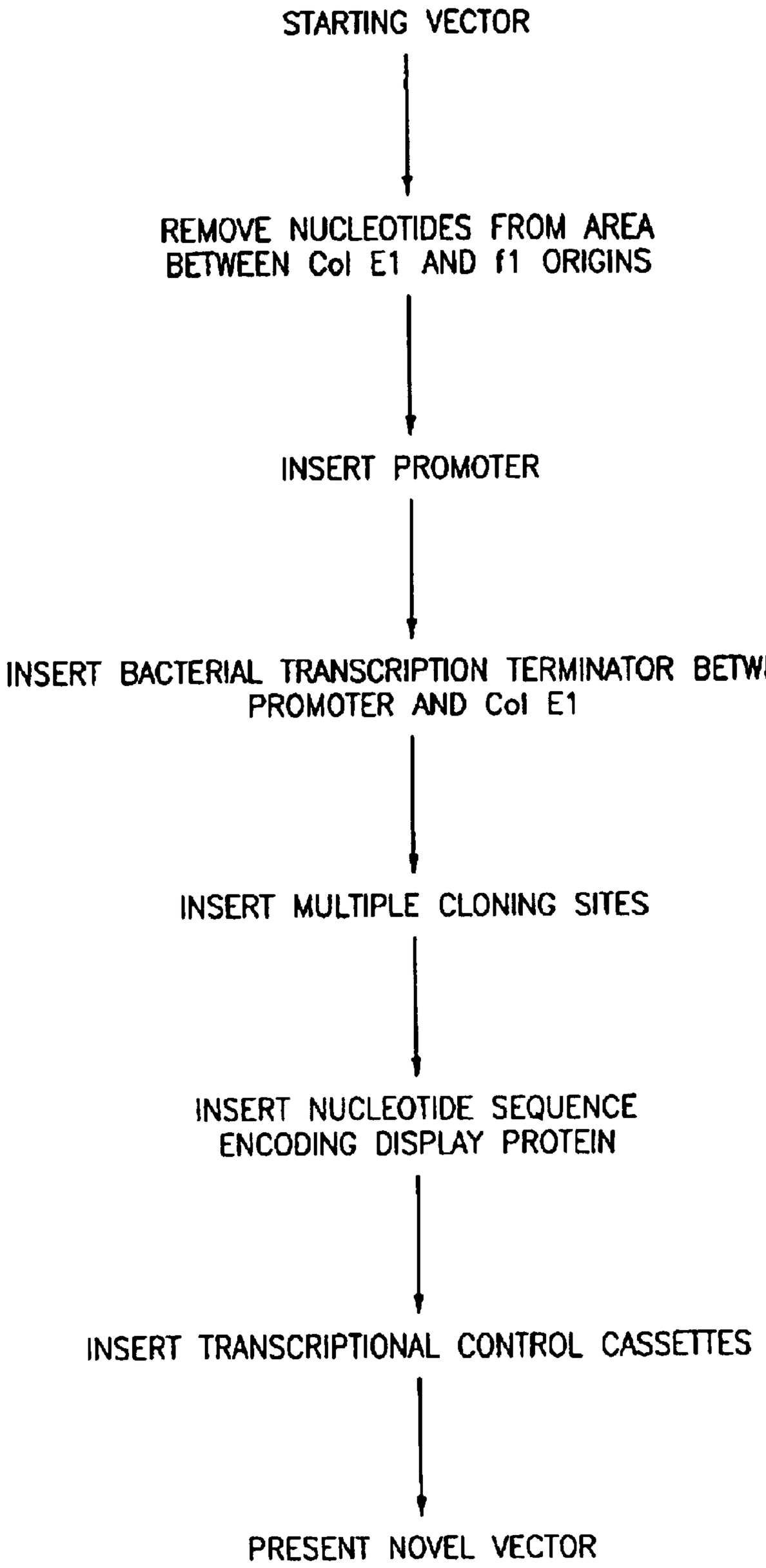

STARTING VECTOR
REMOVE NUCLEOTIDES FROM AREA BETWEEN Col E1 AND f1 ORIGINS
INSERT PROMOTER
INSERT BACTERIAL TRANSCRIPTION TERMINATOR BETWEEN PROMOTER AND Col E1
INSERT MULTIPLE CLONING SITES
INSERT NUCLEOTIDE SEQUENCE ENCODING DISPLAY PROTEIN
INSERT TRANSCRIPTIONAL CONTROL CASSETTES
PRESENT NOVEL VECTOR

FIG. 2

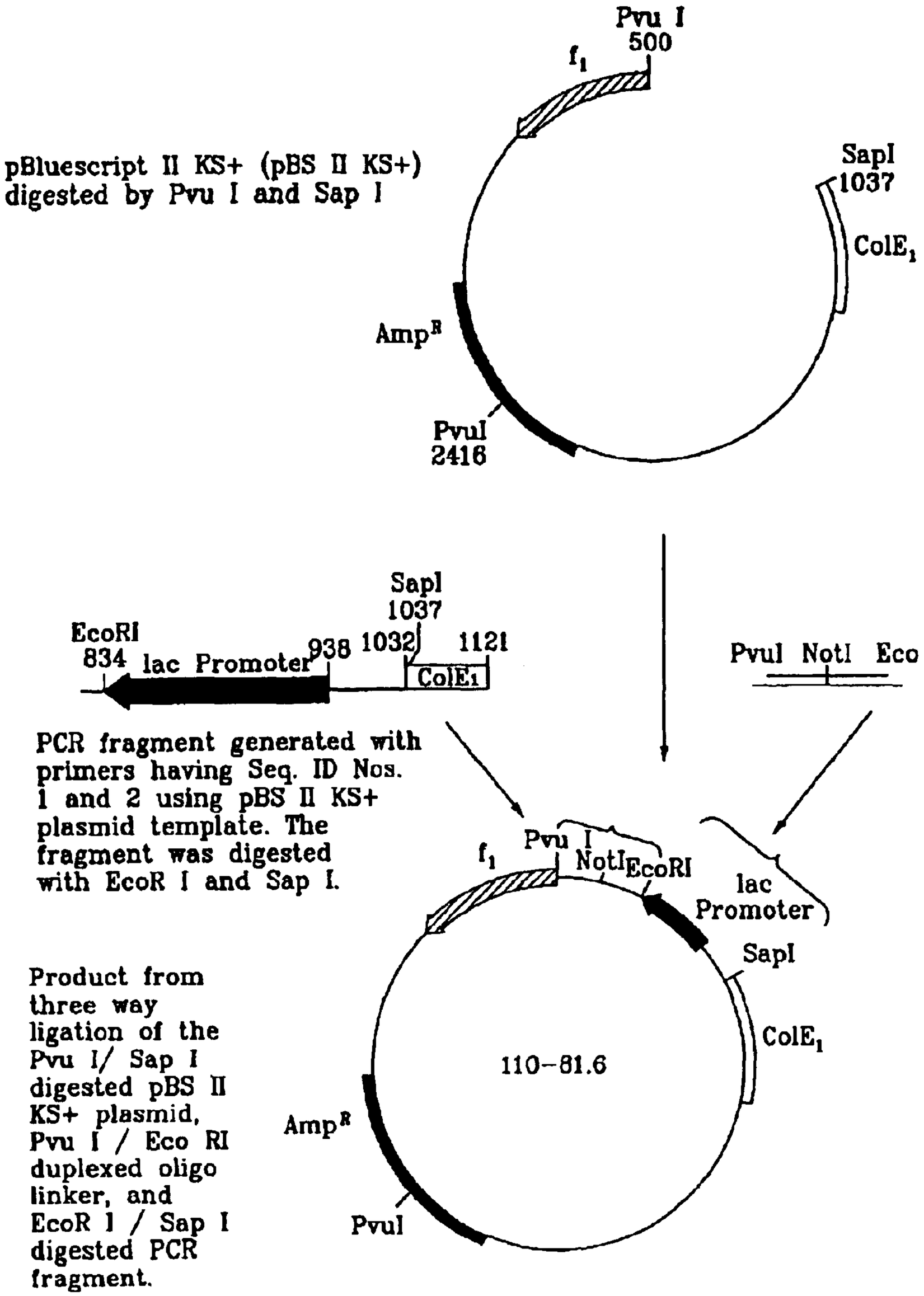

pBluescript II KS+ (pBS II KS+)
digested by Pvu I and Sap I
Pvu I
500
f1
SapI
1037
ColE1
Amp^R
PvuI
2416
EcoRI
834
lac Promoter
938
1032
1121
ColE1
PCR fragment generated with primers having Seq. ID Nos. 1 and 2 using pBS II KS+ plasmid template. The fragment was digested with EcoR I and Sap I.
PvuI NotI Eco
Product from three way ligation of the Pvu I/ Sap I digested pBS II KS+ plasmid, Pvu I / Eco RI duplexed oligo linker, and EcoR I / Sap I digested PCR fragment.
Pvu I
f1
NotI
EcoRI
lac
Promoter
SapI
ColE1
110-81.6
Amp^R
PvuI

FIG. 3

(Seq. ID No. 19)

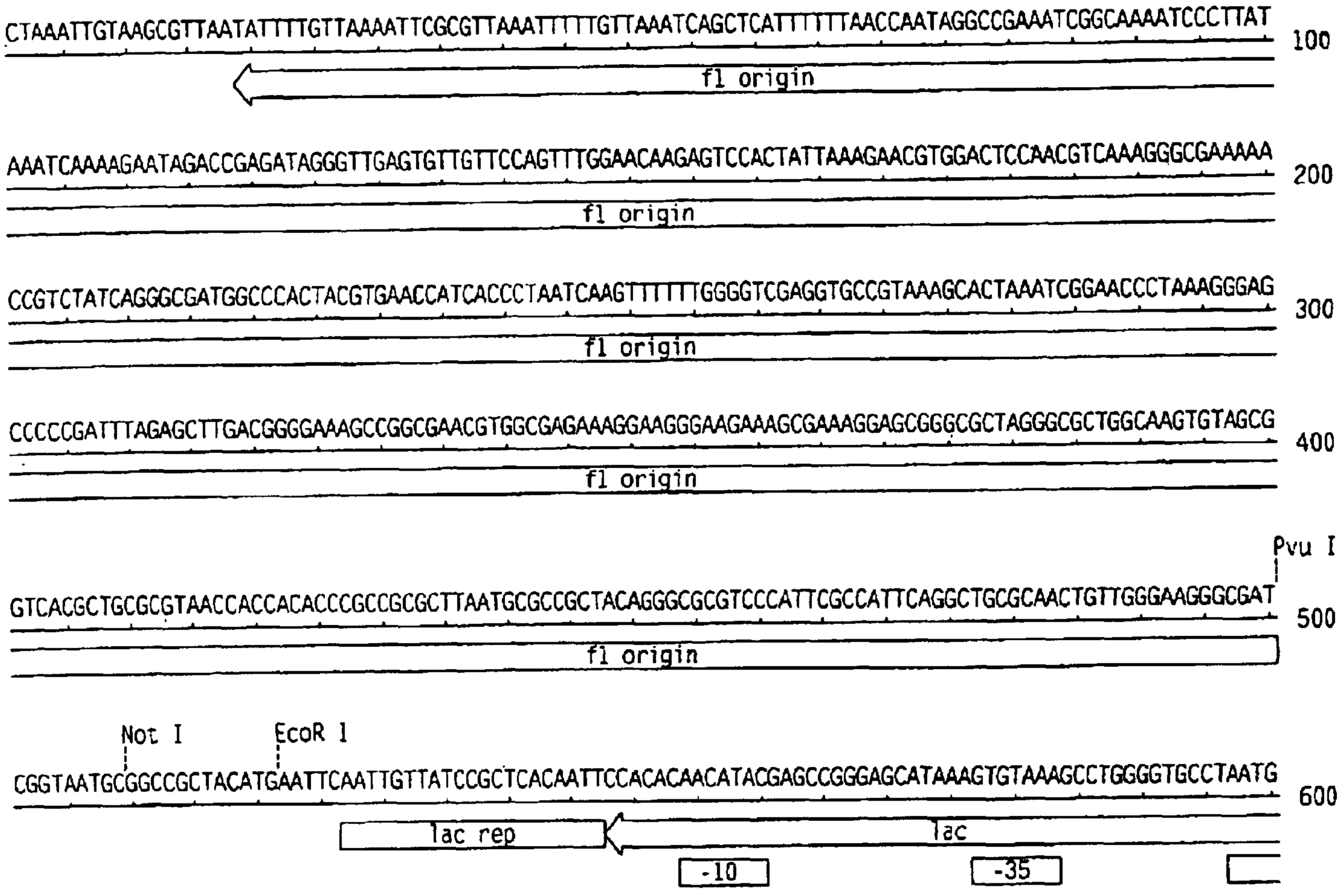
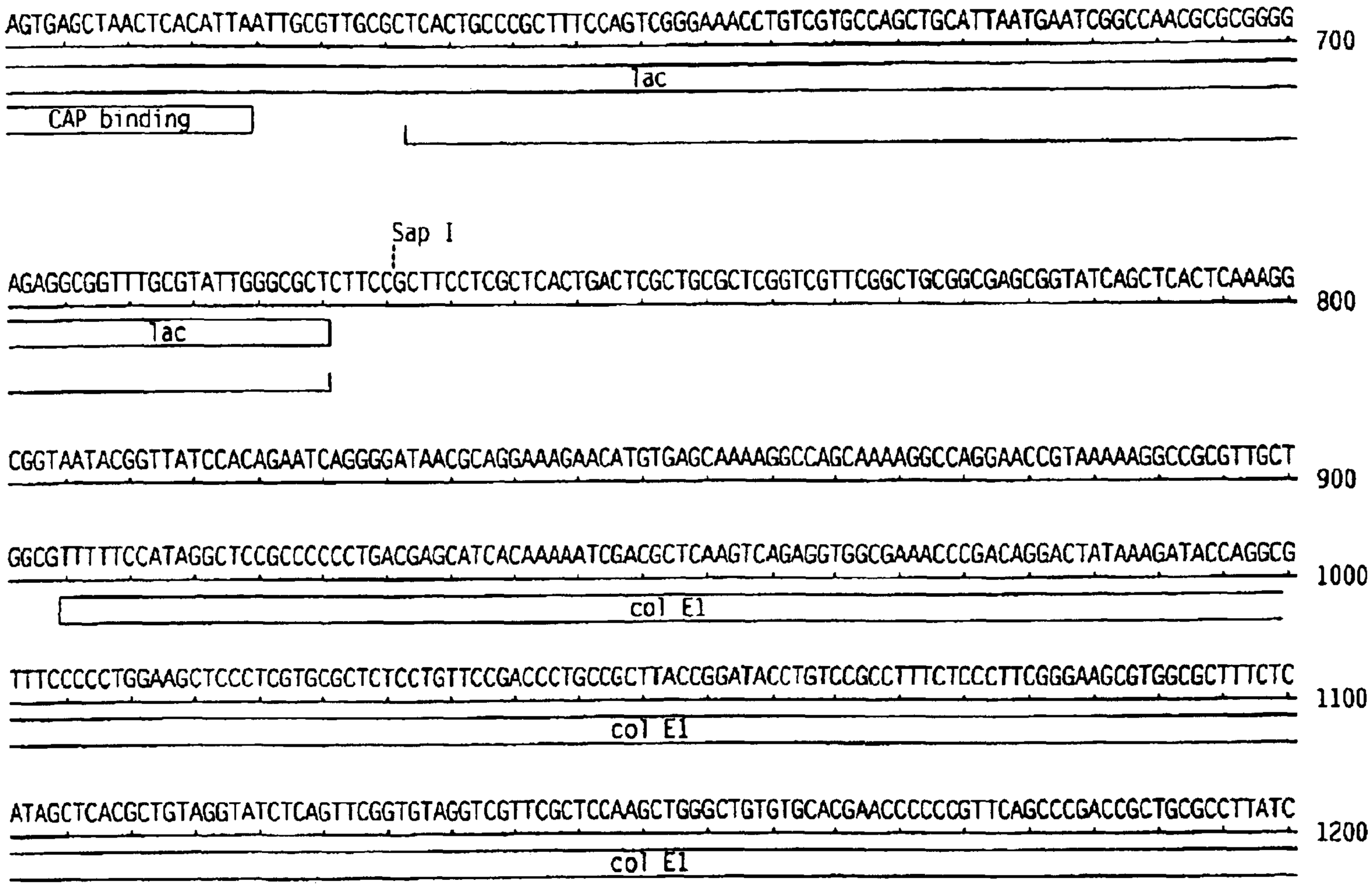


FIG. 4a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700
Tac
CAP binding
Sap I
AGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGG
800
Tac
CGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCT
900
GGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCG
1000
col E1
TTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTC
1100
col E1
ATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATC
1200
col E1

FIG. 4a (Cont.)

CGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGT 1300

col E1

GCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAG 1400

col E1

TTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGA 1500

col E1

TCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATC 1600

CTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAG 1700

beta lactamase

CGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGAT 1800

beta lactamase

ACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCC 1900

beta lactamase

FIG. 4b

ATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCAC 2000

beta lactamase

GCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGG 2100

beta lactamase

Pvu I

TCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACYGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGC 2200

beta lactamase

TTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGC 2300

beta lactamase

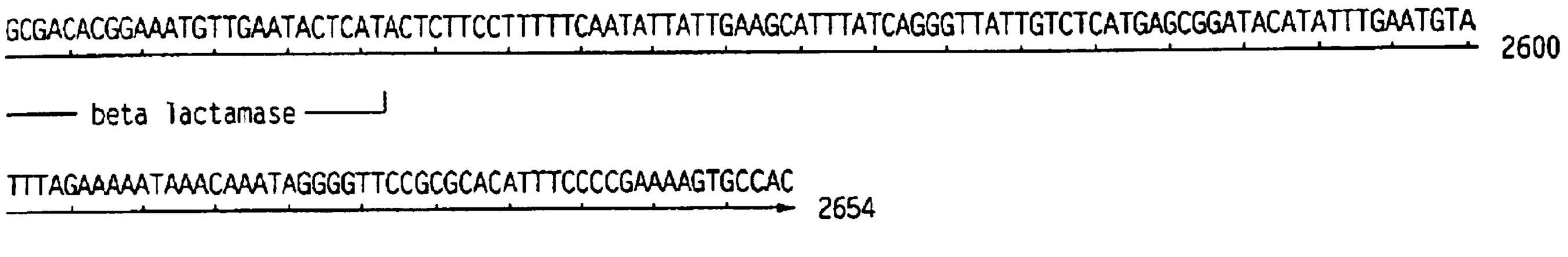
CACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACC 2400

beta lactamase

CACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGG 2500

beta lactamase

FIG. 4b (Cont.)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2600
beta lactamase
TTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCAC
2654

FIG. 4c

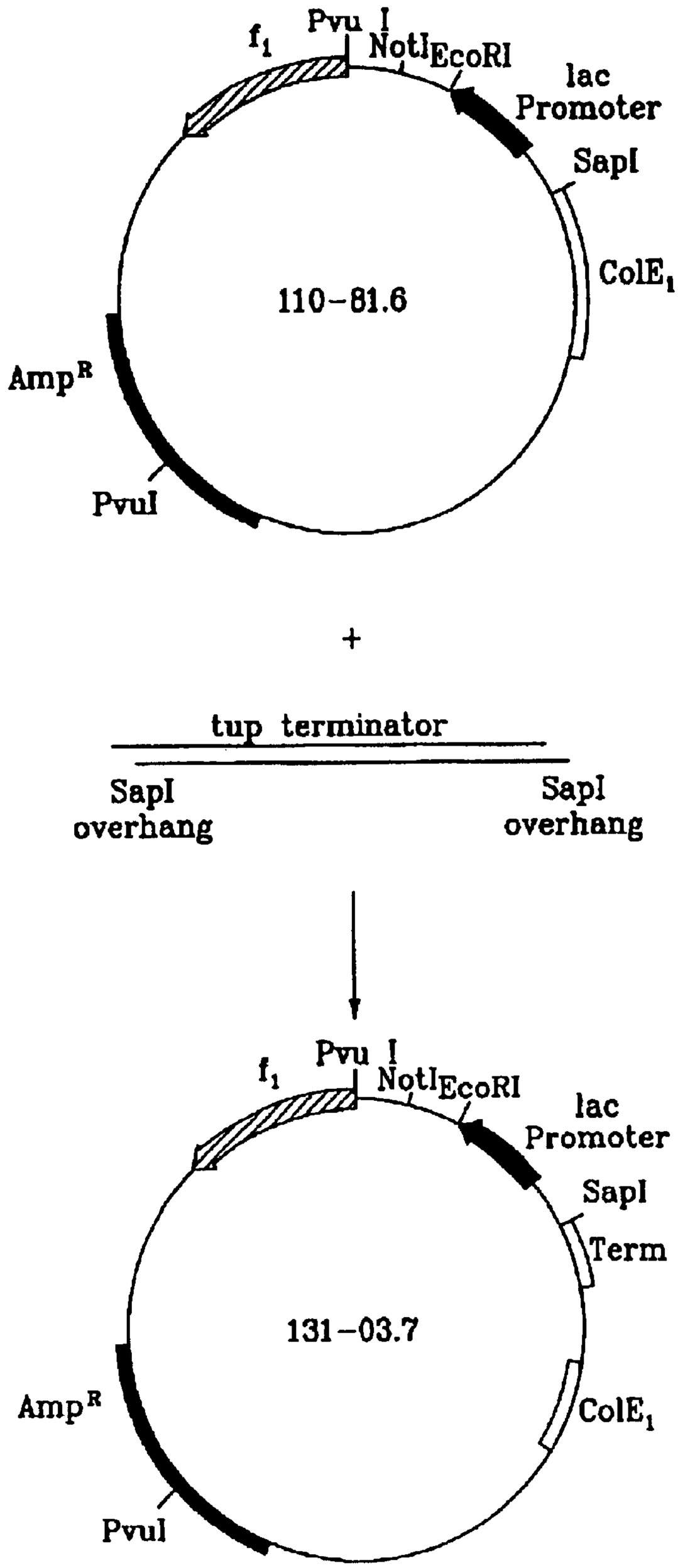

f1
Pvu I
NotI
EcoRI
lac
Promoter
SapI
ColE1
110-81.6
Amp^R
PvuI
+
tup terminator
SapI
overhang
SapI
overhang
f1
Pvu I
NotI
EcoRI
lac
Promoter
SapI
Term
131-03.7
Amp^R
ColE1
PvuI

FIG. 5

(Seq. ID No. 20)

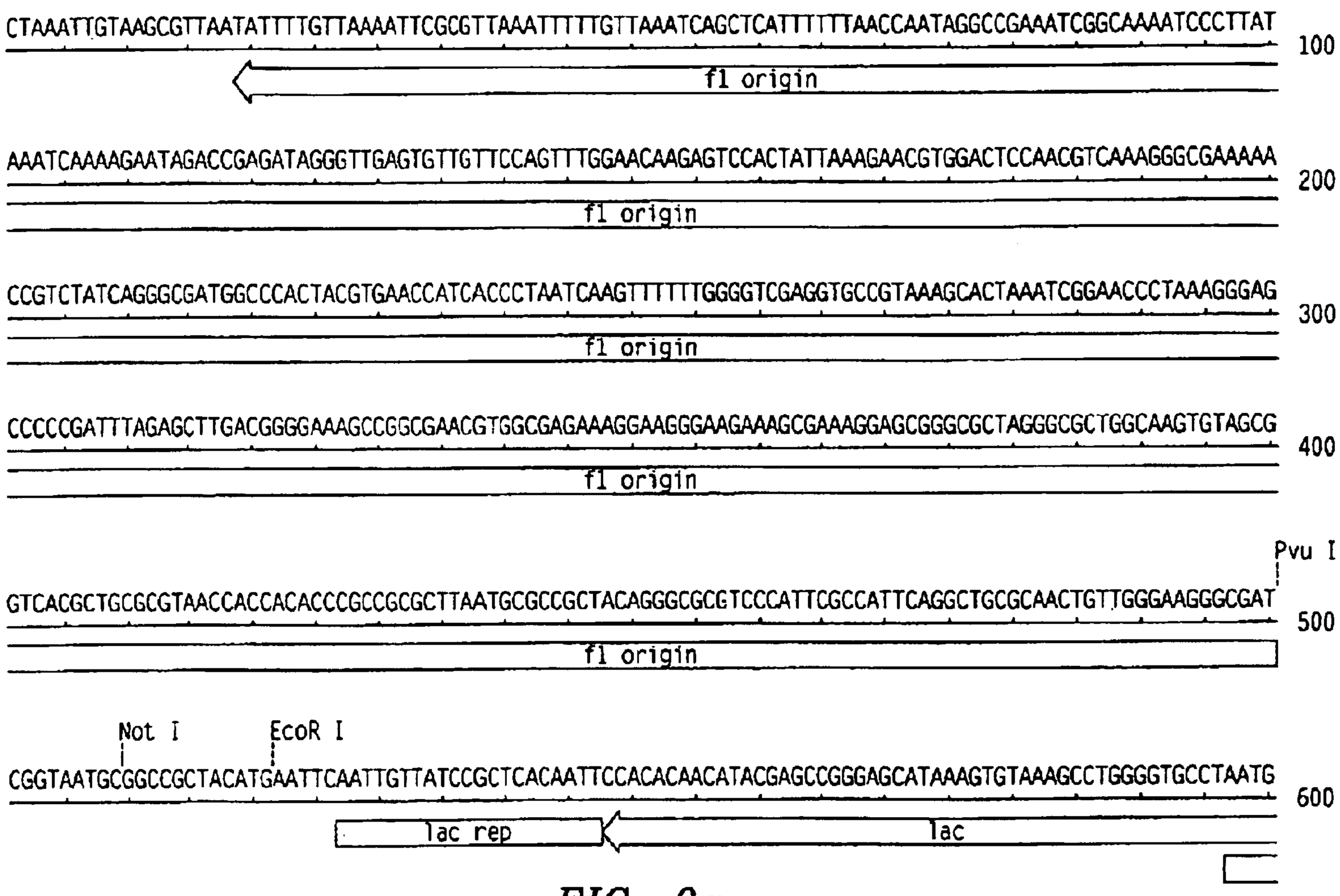


FIG. 6a

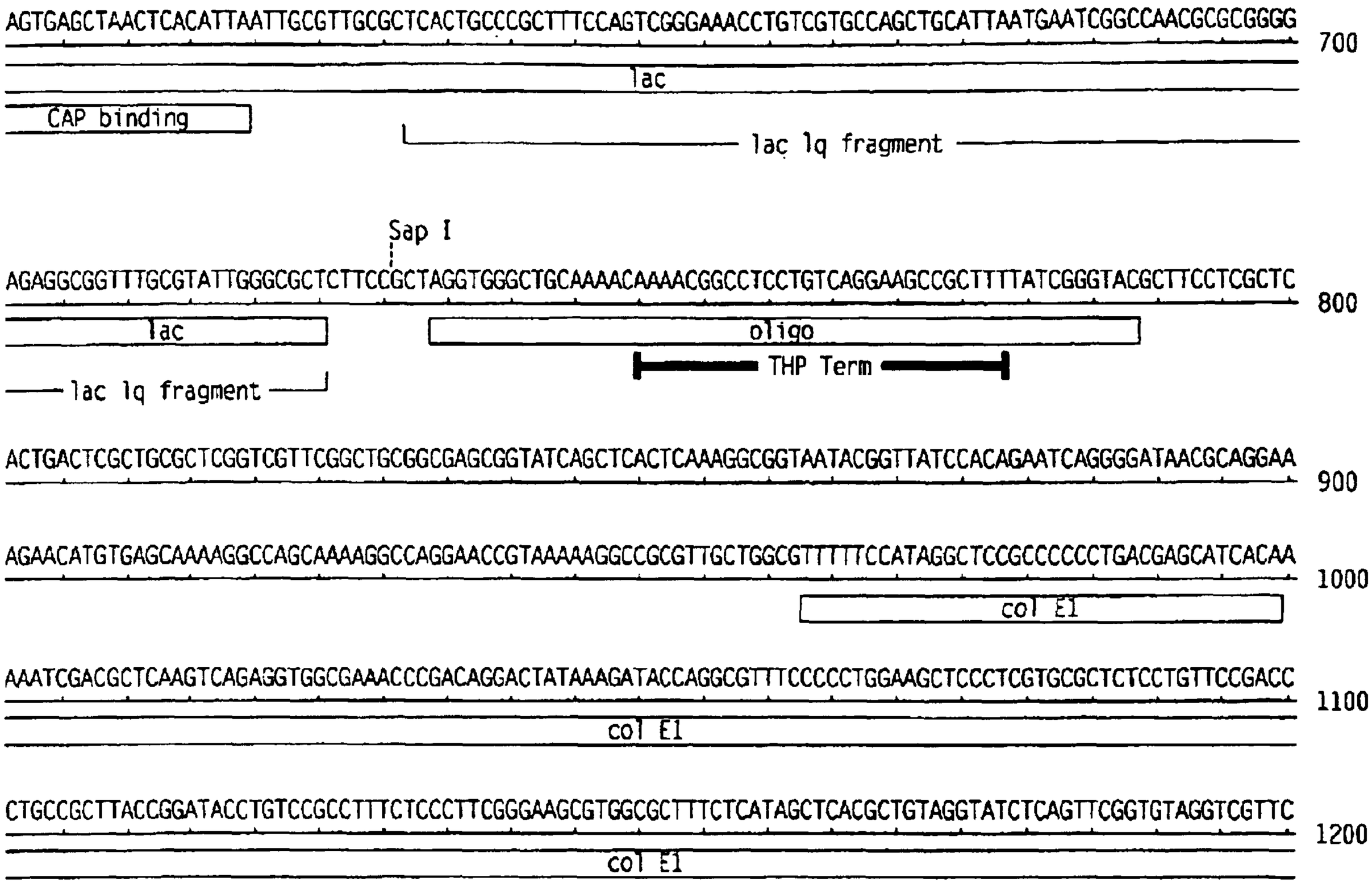

AGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGG
700
lac
CAP binding
lac lq fragment
Sap I
AGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTAGGTGGGCTGCAAAACAAAACGGCCTCCTGTCAGGAAGCCGCTTTTATCGGGTACGCTTCCTCGCTC
800
lac
oligo
THP Term
lac lq fragment
ACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAA
900
AGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAA
1000
col E1
AAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACC
1100
col E1
CTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTC
1200
col E1

FIG. 6a (Cont.)

GCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTT 1300

col E1

ATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACT 1400

col E1

AGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCG 1500

col E1

GTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGA 1600

col E1

AAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATA 1700

TATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCG 1800

FIG. 6b

TCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAAT 1900

AAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGT 2000

TCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAAC 2100

Pvu I

GATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATC 2200

ACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAA 2300

TAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTT 2400

CTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCAC 2500

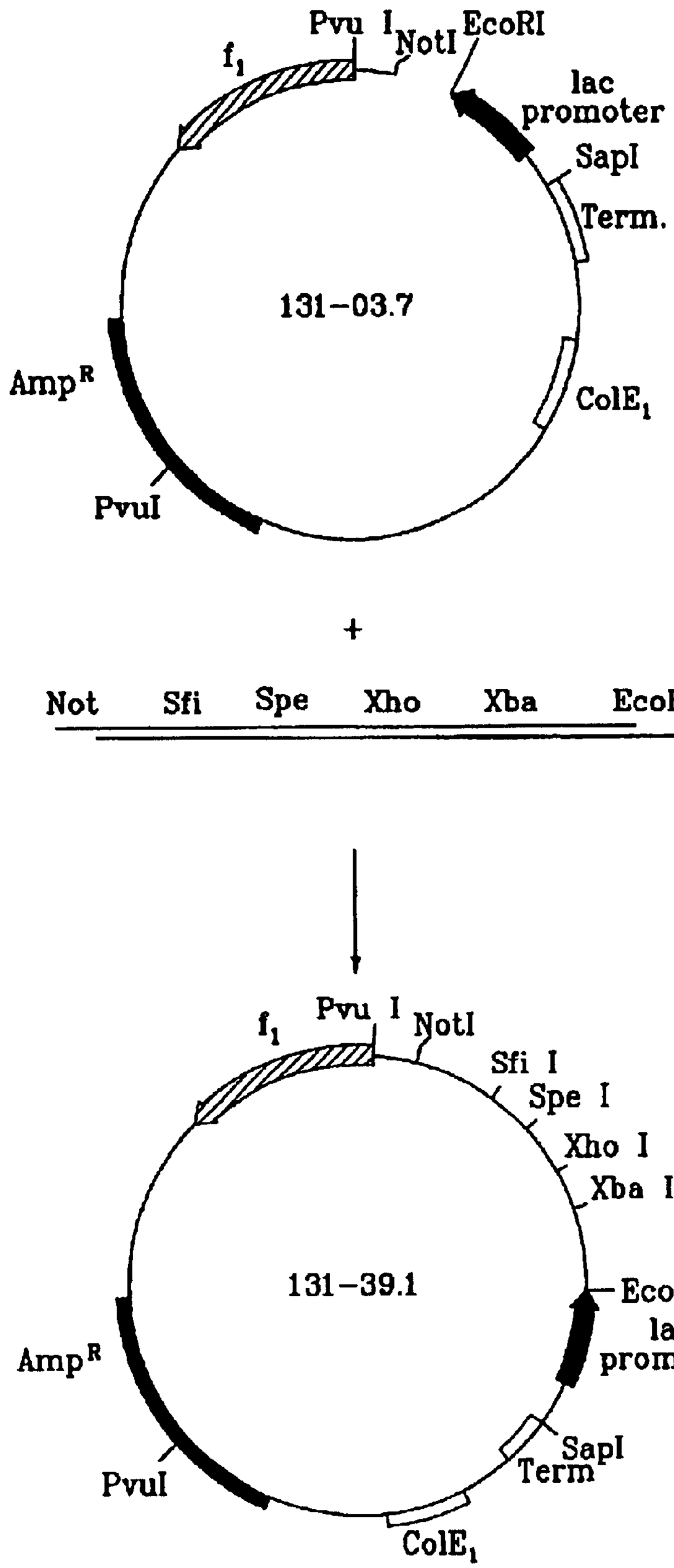
FIG. 6b (Cont.)

CAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAA 2600

TATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCC 2700

GAAAAGTGCCAC 2712

FIG. 6c



131-03.7 digested Not I and EcoRI

FIG. 7

(Seq. ID No. 21)

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GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCAC 200

beta lactamase

CCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTT 300

beta lactamase

TTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCG 400

beta lactamase

CCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCC 500

beta lactamase

Pvu I

ATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAA 600

beta lactamase

FIG. 8a

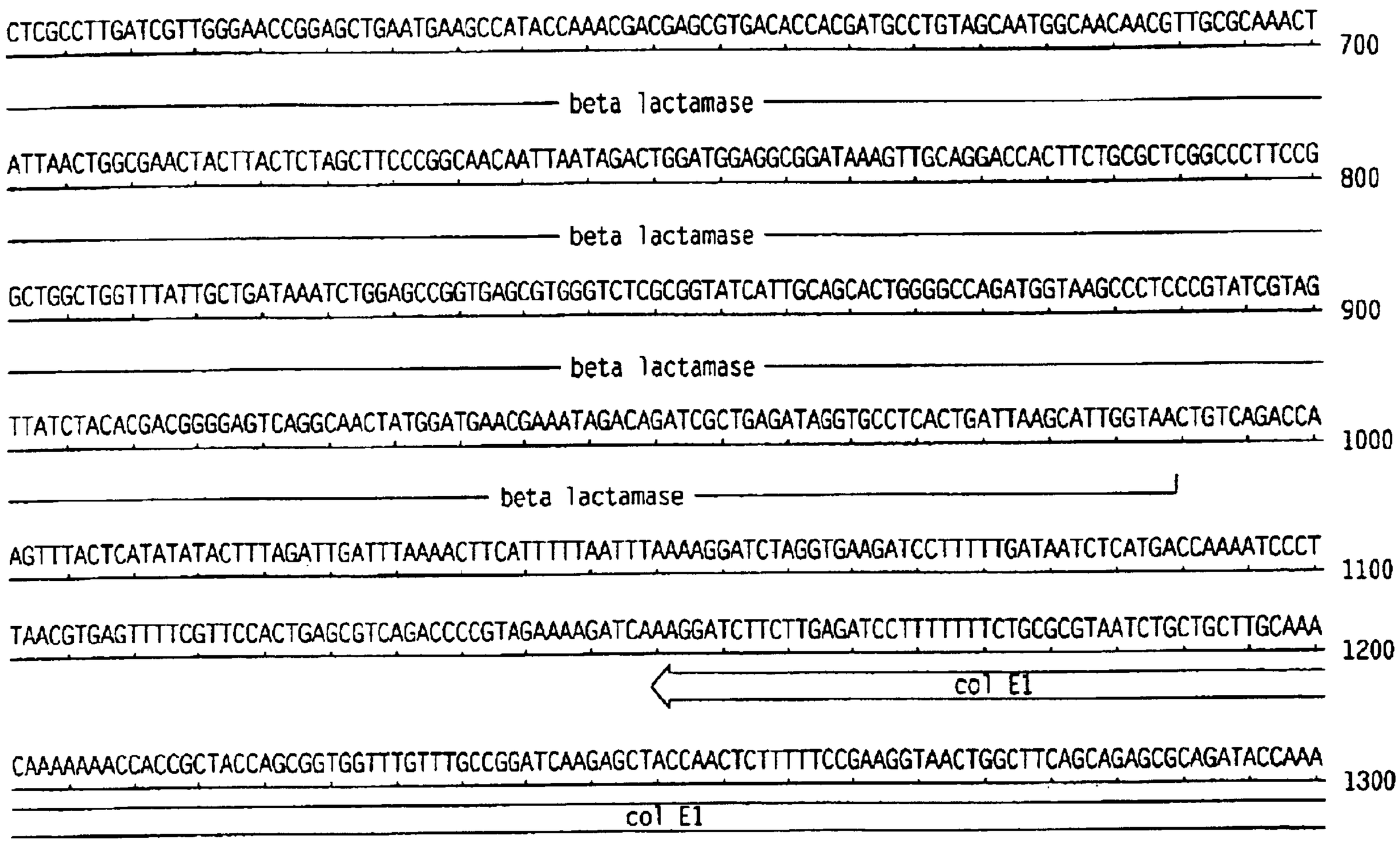
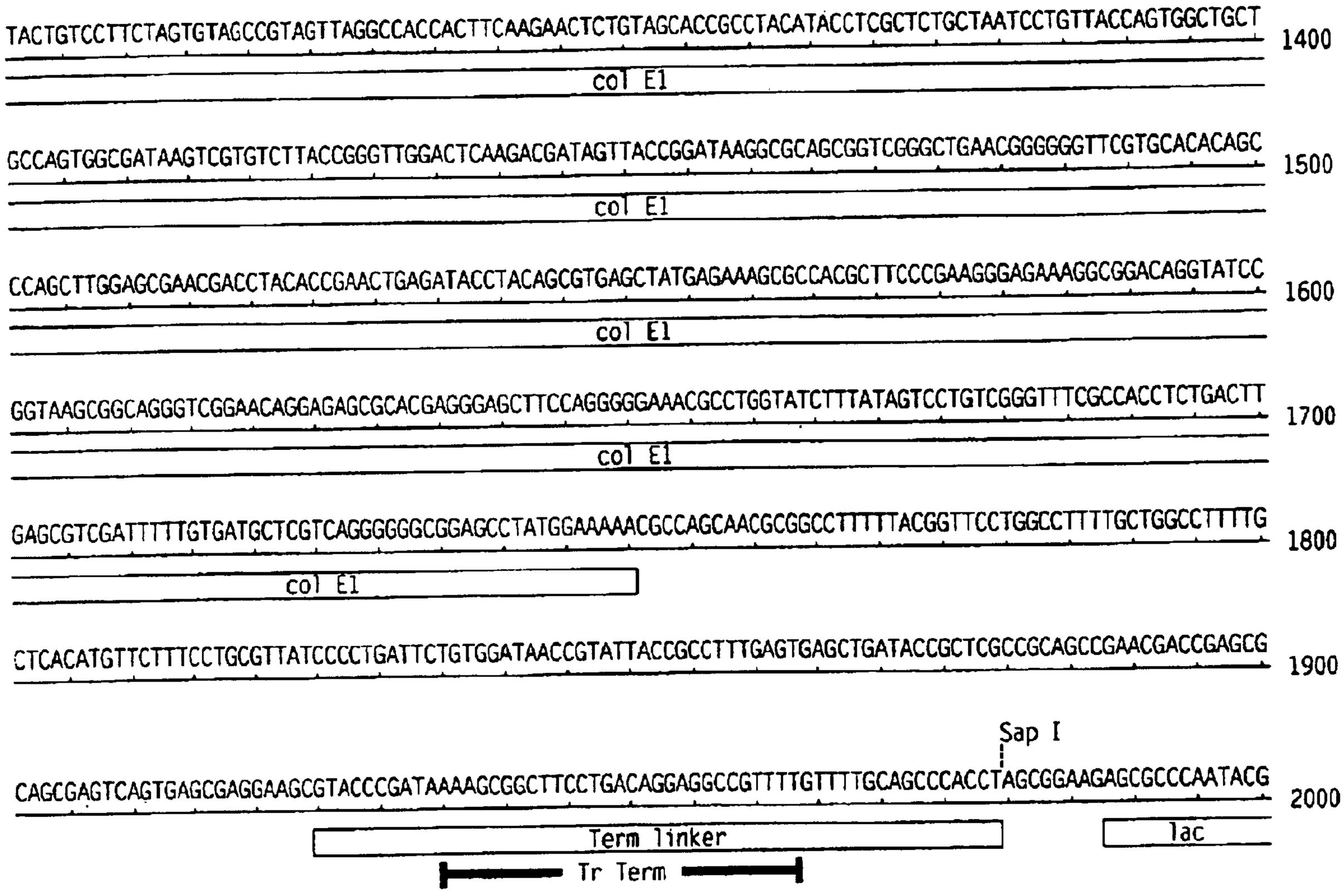

CTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACT
700
beta lactamase
ATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCG
800
beta lactamase
GCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAG
900
beta lactamase
TTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCA
1000
beta lactamase
AGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCT
1100
TAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAA
1200
col E1
CAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAA
1300
col E1

FIG. 8a (Cont.)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1400
col E1
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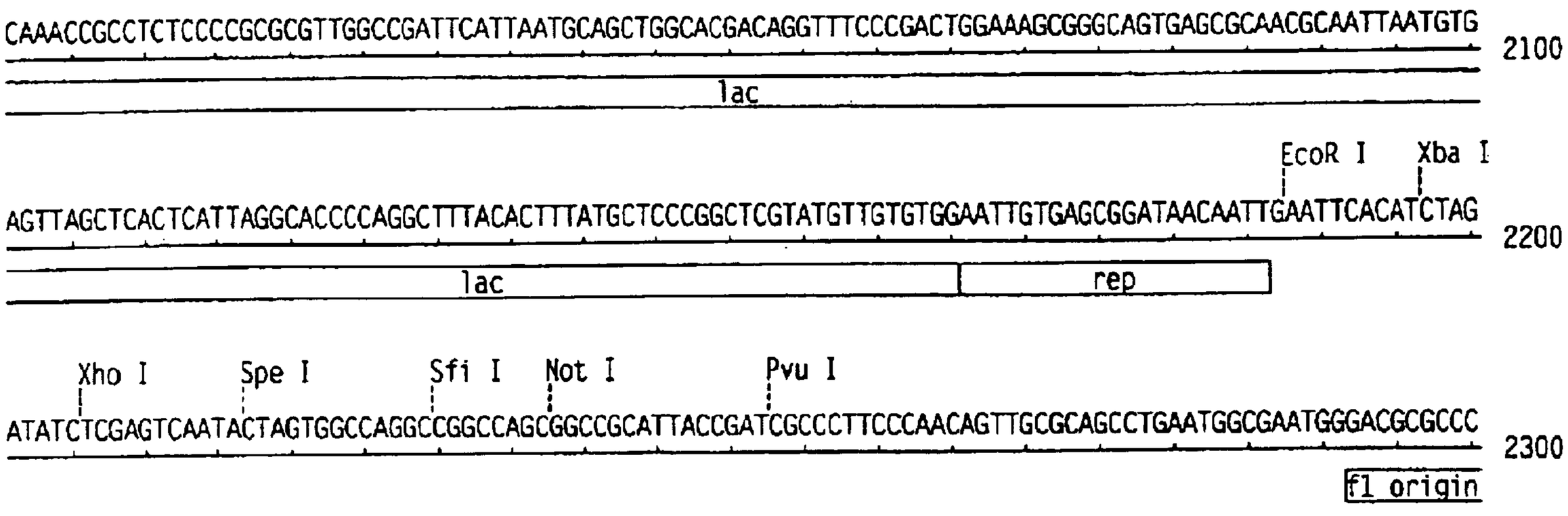
1500
col E1
CCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCC
1600
col E1
GGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTT
1700
col E1
GAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTG
1800
col E1
CTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCG
1900
Sap I
CAGCGAGTCAGTGAGCGAGGAAGCGTACCCGATAAAAGCGGCTTCCTGACAGGAGGCCGTTTTGTTTTGCAGCCCACCTAGCGGAAGAGCGCCCAATACG
2000
Term linker
lac
Tr Term

FIG. 8b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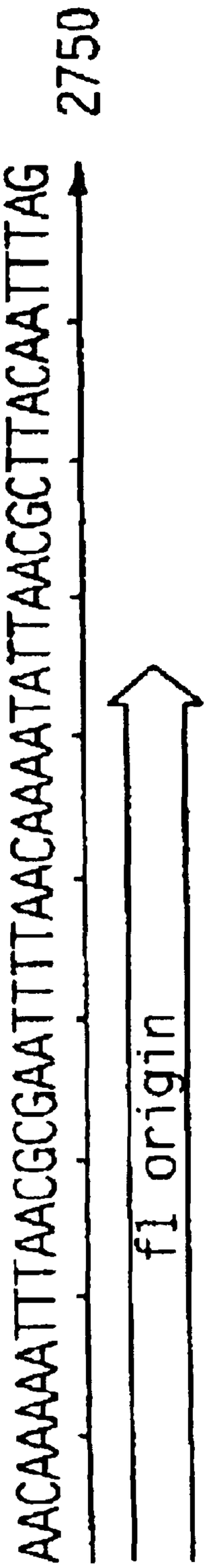
2100
lac
EcoR I
Xba I
AGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTCCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTGAATTCACATCTAG
2200
lac
rep
Xho I
Spe I
Sfi I
Not I
Pvu I
ATATCTCGAGTCAATACTAGTGGCCAGGCCGGCCAGCGGCCGCATTACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGCCC
2300
f1 origin
TGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTT
2400
f1 origin
CCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAA
2500
f1 origin
ACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTG
2600
f1 origin
TTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTT
2700
f1 origin

FIG. 8b (Cont.)


2750
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fl origin

FIG. 8c

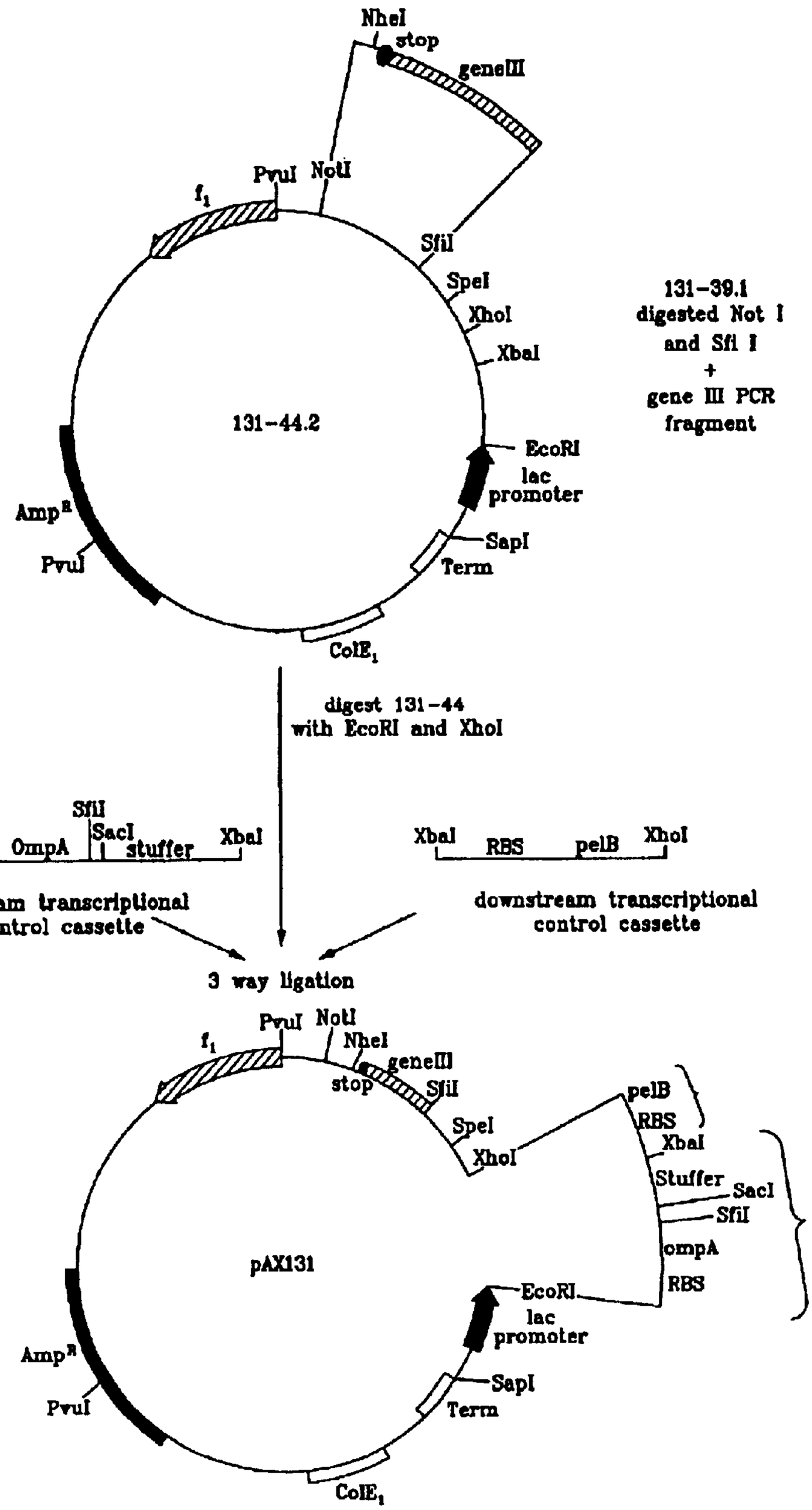
NheI
stop
geneIII
PvuI
NotI
f1
SfiI
SpeI
XhoI
XbaI
131-44.2
EcoRI
lac
promoter
SapI
Term
ColE1
Amp^R
PvuI
131-39.1
digested Not I
and Sfi I
+
gene III PCR
fragment
digest 131-44
with EcoRI and XhoI
SfiI
EcoRI
RBS
OmpA
SacI
stuffer
XbaI
XbaI
RBS
pelB
XhoI
upstream transcriptional
control cassette
downstream transcriptional
control cassette
3 way ligation
PvuI
NotI
NheI
f1
stop
geneIII
SfiI
SpeI
XhoI
pelB
RBS
XbaI
Stuffer
SacI
SfiI
ompA
RBS
pAX131
EcoRI
lac
promoter
SapI
Term
ColE1
Amp^R
PvuI

FIG. 9

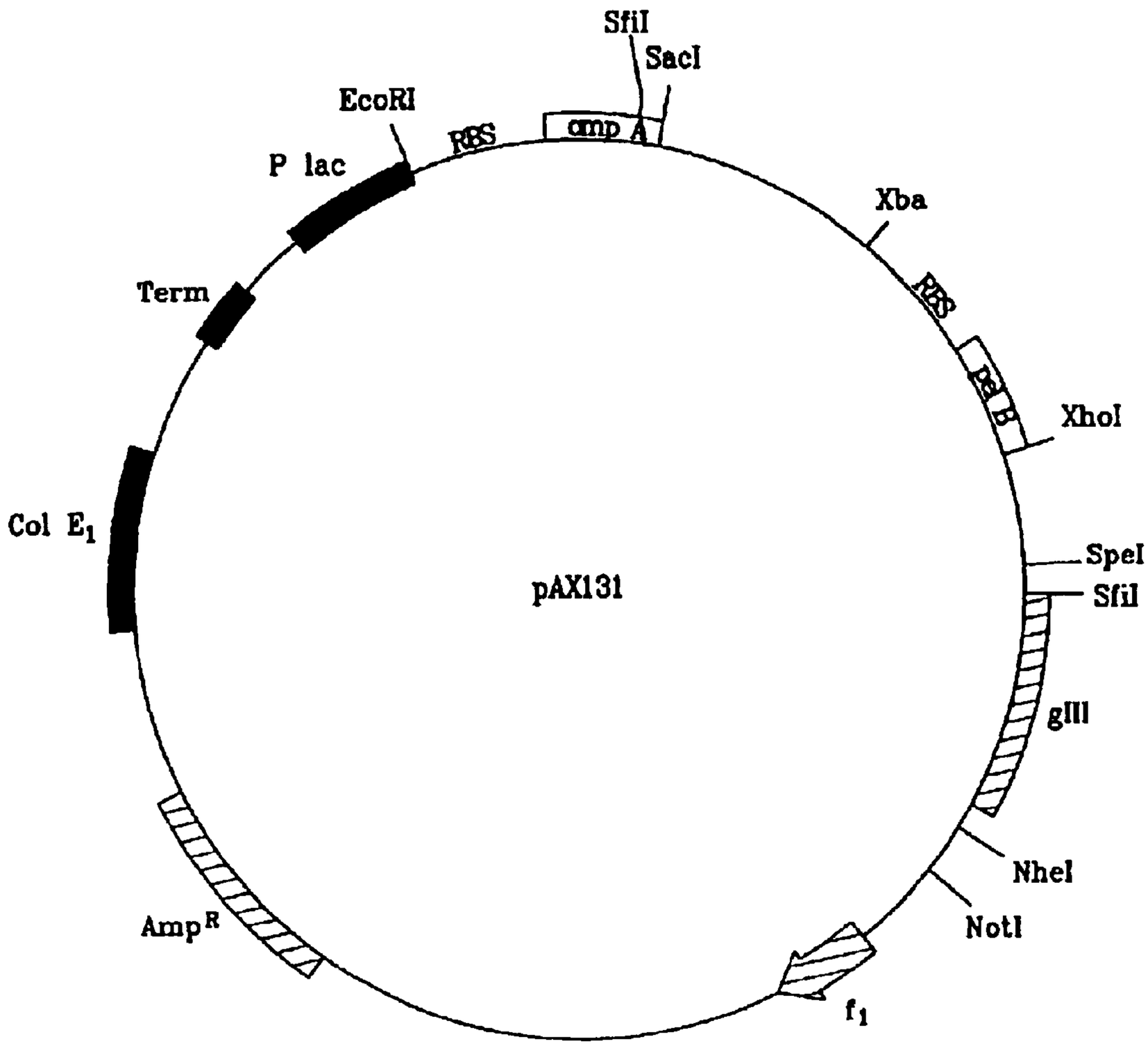
SfiI
SacI
EcoRI
RBS
amp A
P lac
Xba
Term
RBS
peL B
XhoI
Col E1
SpeI
SfiI
pAX131
gIII
NheI
NotI
AmpR
f1

FIG. 10

Seq. ID No. 18

GTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAAT 100

Ear I

GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCAC 200

beta lactamase

CCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTT 300

beta lactamase

TTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCG 400

beta lactamase

CCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCC 500

beta lactamase

ATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAA 600

beta lactamase

FIG. 11a

CTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACT 700

beta lactamase

ATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCG 800

beta lactamase

GCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAG 900

beta lactamase

TTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCA 1000

beta lactamase

AGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCAAAATCCCT 1100

TAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAA 1200

col E1

CAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAA 1300

col E1

FIG. 11α (Cont.)

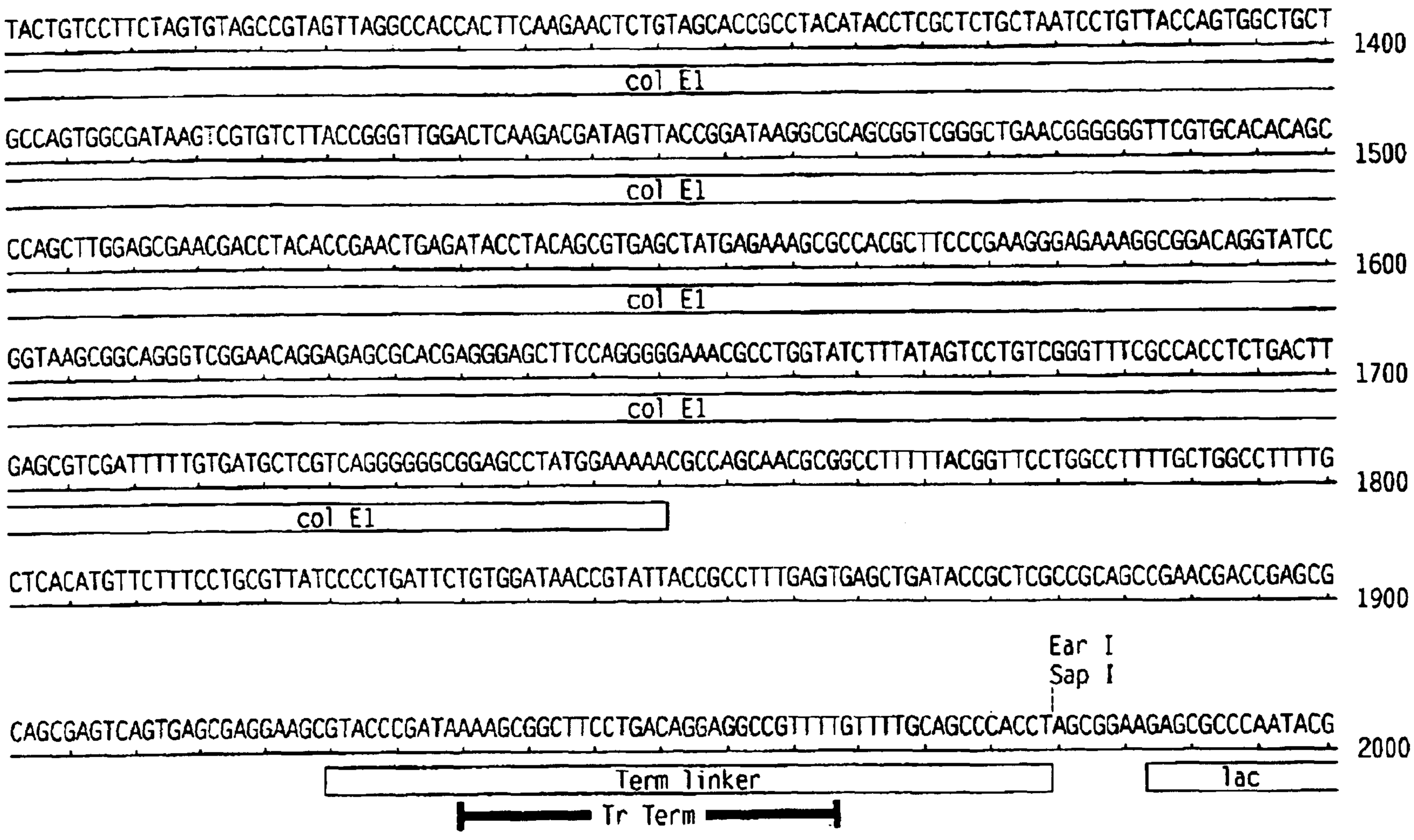
TACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCT
1400
col E1
GCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGC
1500
col E1
CCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCC
1600
col E1
GGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTT
1700
col E1
GAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTG
1800
col E1
CTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCG
1900
Ear I
Sap I
CAGCGAGTCAGTGAGCGAGGAAGCGTACCCGATAAAAGCGGCTTCCTGACAGGAGGCCGTTTTGTTTTGCAGCCCACCTAGCGGAAGAGCGCCCAATACG
2000
Term linker
lac
Tr Term

FIG. 11b

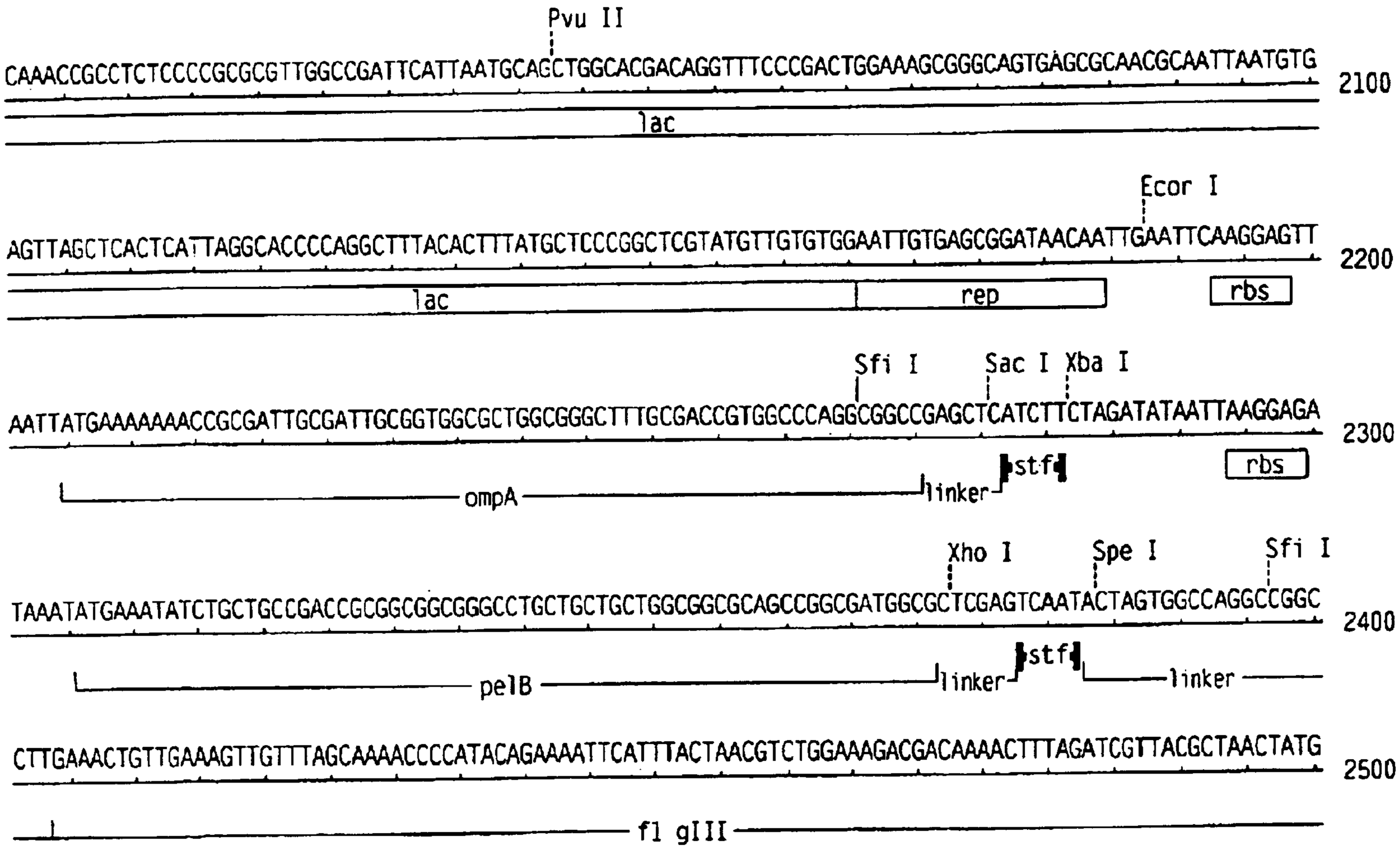
Pvu II
CAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTG
2100
lac
Ecor I
AGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTCCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTGAATTCAAGGAGTT
2200
lac
rep
rbs
Sfi I
Sac I
Xba I
AATTATGAAAAAAACCGCGATTGCGATTGCGGTGGCGCTGGCGGGCTTTGCGACCGTGGCCCAGGCGGCCGAGCTCATCTTCTAGATATAATTAAGGAGA
2300
rbs
stf
ompA
linker
Xho I
Spe I
Sfi I
TAAATATGAAATATCTGCTGCCGACCGCGGCGGCGGGCCTGCTGCTGCTGGCGGCGCAGCCGGCGATGGCGCTCGAGTCAATACTAGTGGCCAGGCCGGC
2400
stf
pelB
linker
linker
CTTGAAACTGTTGAAAGTTGTTTAGCAAAACCCCATACAGAAAATTCATTTACTAACGTCTGGAAAGACGACAAAACTTTAGATCGTTACGCTAACTATG
2500
fl gIII

FIG. 11b (Cont.)

AGGGCTGTCTGTGGAATGCTACAGGCGTTGTAGTTTGTACTGGTGACGAAACTCAGTGTTACGGTACATGGGTTCCTATTGGGCTTGCTATCCCTGAAAA 2600

f1 gIII

TGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGTGGCGGTACTAAACCTCCTGAGTACGGTGATACACCTATTCCGGGCTAT 2700

f1 gIII

ACTTATATCAACCCTCTCGACGGCACTTATCCGCCTGGTACTGAGCAAAACCCCGCTAATCCTAATCCTTCTCTTGAGGAGTCTCAGCCTCTTAATACTT 2800

f1 gIII

TCATGTTTCAGAATAATAGGTTCCGAAATAGGCAGGGGGCATTAACTGTTTATACGGGCACTGTTACTCAAGGCACTGACCCCGTTAAAACTTATTACCA 2900

f1 gIII

GTACACTCCTGTATCATCAAAAGCCATGTATGACGCTTACTGGAACGGTAAATTCAGAGACTGCGCTTTCCATTCTGGCTTTAATGAGGATCCATTCGTT 3000

f1 gIII

TGTGAATATCAAGGCCAATCGTCTGACCTGCCTCAACCTCCTGTTAATGCTGGCGGCGGCTCTGGTGGTGGTTCTGGTGGCGGCTCTGAGGGTGGTGGCT 3100

f1 gIII

CTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGTGGCGGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCAAACGCTAA 3200

f1 gIII

FIG. 11c

TAAGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTCTGACGCTAAAGGCAAACTTGATTCTGTCGCTACTGATTACGGTGCTGCTATCGAC 3300

f1 gIII

GGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGTGATTTTGCTGGCTCTAATTCCCAAATGGCTCAAGTCGGTGACGGTGATA 3400

f1 gIII

ATTCACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGAATGTCGCCCTTTTGTCTTTGGCGCTGGTAAACCATATGAATT 3500

f1 gIII

TTCTATTGATTGTGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCACCTTTATGTATGTATTTTCGACGTTTGCTAACATA 3600

f1 gIII

Not I

CTGCGTAATAAAGAGTCTTAAGCTAGCTAAGCGGCCGCATTACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGCCCTGTAG 3700

f1 ori

f1 gIII

CGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTT 3800

f1 ori

FIG. 11c (Cont.)

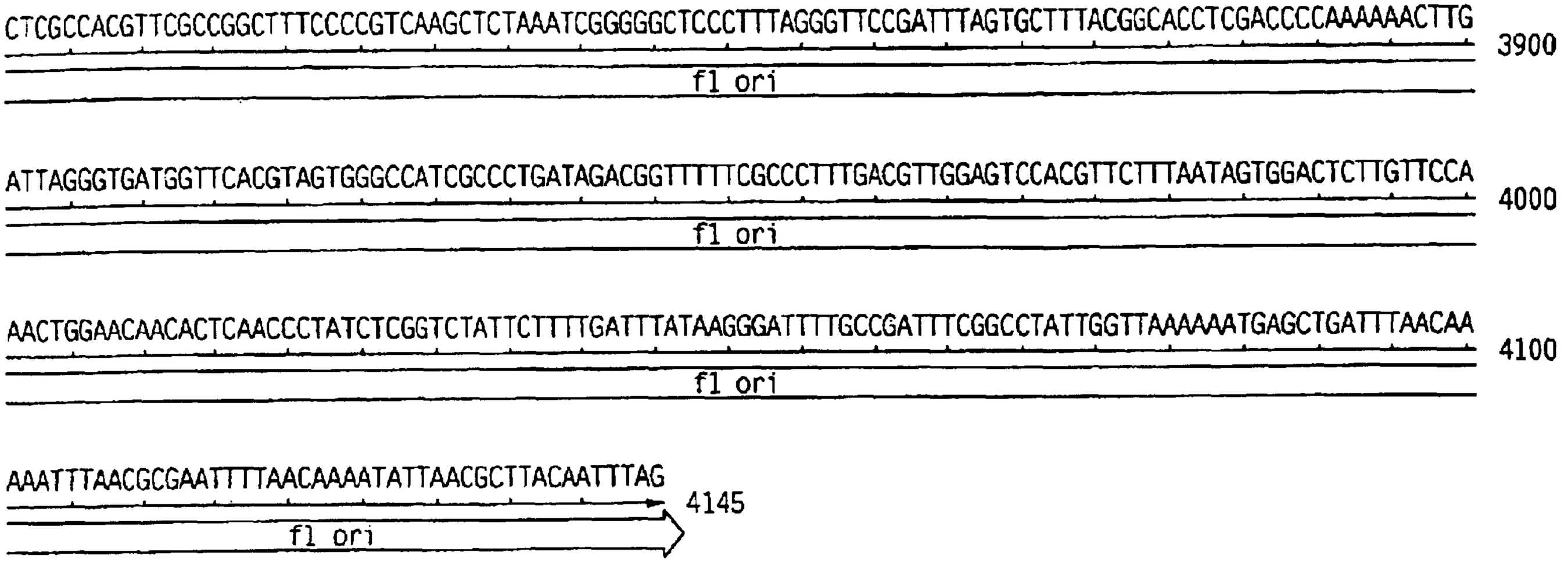
CTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTG
3900
f1 ori
ATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCA
4000
f1 ori
AACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAA
4100
f1 ori
AAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTAG
4145
f1 ori

FIG. 11d

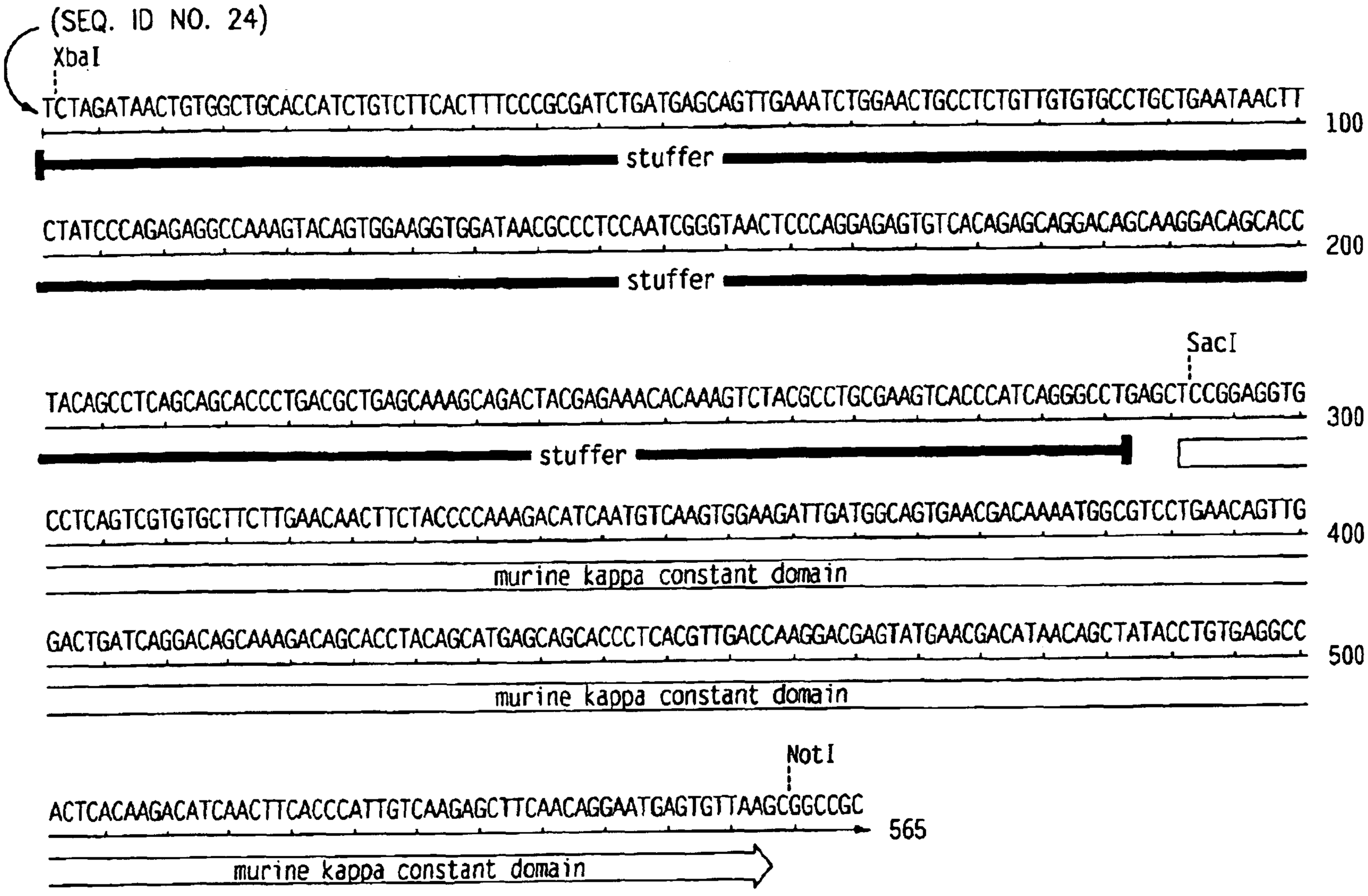
(SEQ. ID NO. 24)
XbaI
TCTAGATAACTGTGGCTGCACCATCTGTCTTCACTTTCCCGCGATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTT
100
stuffer
CTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACC
200
stuffer
SacI
TACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCCGGAGGTG
300
stuffer
CCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTG
400
murine kappa constant domain
GACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCC
500
murine kappa constant domain
NotI
ACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAAGCGGCCGC
565
murine kappa constant domain

FIG. 12A

(SEQ. ID NO. 25)

XbaI

CTCGAGCTGATGAGCCATGGAAGCTGTGTCGCCTGCACCAGGCTCCCACGGCTCGTGGTGCGGTGCGCTTCTGGTGTTCGCTGCCTACAGCCGACACGTC 100

stuffer

GAGCTTCGTGCCCCTAGAGTTGCGCGTCACAGCAGCCTCCGGCGCTCCGCGATATCACCGTGTCATCCACATCAATGAAGTAGTGCTCCTAGACGCCCCC 200

stuffer

GTGGGGCTGGTGGCGCGGTTGGCTGACGAGAGCGGCCACGTAGTGTTGCGCTGGCTCCCGCCGCCTGAGACACCCATGACGTCTCACATCCGCTACGAGG 300

stuffer

TGGACGTCTCGGCCGGCAACGGCGCAGGGAGCGTACAGAGGGTGGAGATCCTGGAGGGCCGCACCGAGTGTGTGCTGAGCAACCTGCGGGGCCGGACGCG 400

stuffer

CTACACCTTCGCCGTCCGCGCGCGTATGGCTGAGCCGAGCTTCGGCGGCTTCTGGAGCGCCTGGTCGGAGCCTGTGTCGCTGCTGACGCCTAGCGACCTG 500

stuffer

GACCCCCTCATCCTGACGCTCTCCCTCATCCTCGTGGTCATCCTGGTGCTGCTGACCGTGCTCGCGCTGCTCTCCCACCGCCGGGCTCTGAAGCAGAAGA 600

stuffer

TCTGGCCTGGCATCCCGAGCCCAGAGAGCGAGTTTGAAGGCCTCTTCACCACCCACAAGGGTAACTTCCAGCTGTGGCTGTACCAGAATGATGGCTGCCT 700

stuffer

FIG. 12B

GTGGTGGAGCCCCTGCACCCCCTTCACGGAGGACCCACCTGCTTCCCTGGAAGTCCTCTCAGAGCGCTGCTGGGGGACGATGCAGGCAGTGGAGCCGGGG 800

stuffer

ApaI EcoRI

ACAGATGATGAGGGCCCTTTTCCCCCTCGTCTCCTGTGAGAATTCCCCGTCGGATACGAGCAGCGTGGCCGTTGGCTGCCTCGCACAGGACTTCCTTCCC 900

stuffer

human IgM CH1 domain

GACTCCATCACTTTCTCCTGGAAATACAAGAACAACTCTGACATCAGCAGCACCCGGGGCTTCCCATCAGTCCTGAGAGGGGGCAAGTACGCAGCCACCT 1000

human IgM CH1 domain

CACAGGTGCTGCTGCCTTCCAAGGACGTCATGCAGGGCACAGACGAACACGTGGTGTGCAAAGTCCAGCACCCCAACGGCAACAAAGAAAAGAACGTGCC 1100

human IgM CH1 domain

SpeI

TCTTCCAGTGATTGCTGAGCTGCCTACTAGT 1131

human IgM CH1 domain

FIG. 12B (Cont.)

(SEQ. ID NO. 26)

XhoI

CTCGAGCTGATGAGCCATGGAAGCTGTGTCGCCTGCACCAGGCTCCCACGGCTCGTGGTGCGGTGCGCTTCTGGTGTTCGCTGCCTACAGCCGACACGTC 100

stuffer

GAGCTTCGTGCCCCTAGAGTTGCGCGTCACAGCAGCCTCCGGCGCTCCGCGATATCACCGTGTCATCCACATCAATGAAGTAGTGCTCCTAGACGCCCCC 200

stuffer

GTGGGGCTGGTGGCGCGGTTGGCTGACGAGAGCGGCCACGTAGTGTTGCGCTGGCTCCCGCCGCCTGAGACACCCATGACGTCTCACATCCGCTACGAGG 300

stuffer

TGGACGTCTCGGCCGGCAACGGCGCAGGGAGCGTACAGAGGGTGGAGATCCTGGAGGGCCGCACCGAGTGTGTGCTGAGCAACCTGCGGGGCCGGACGCG 400

stuffer

CTACACCTTCGCCGTCCGCGCGCGTATGGCTGAGCCGAGCTTCGGCGGCTTCTGGAGCGCCTGGTCGGAGCCTGTGTCGCTGCTGACGCCTAGCGACCTG 500

stuffer

GACCCCCTCATCCTGACGCTCTCCCTCATCCTCGTGGTCATCCTGGTGCTGCTGACCGTGCTCGCGCTGCTCTCCCACCGCCGGGCTCTGAAGCAGAAGA 600

stuffer

TCTGGCCTGGCATCCCGAGCCCAGAGAGCGAGTTTGAAGGCCTCTTCACCACCCACAAGGGTAACTTCCAGCTGTGGCTGTACCAGAATGATGGCTGCCT 700

stuffer

FIG. 12C

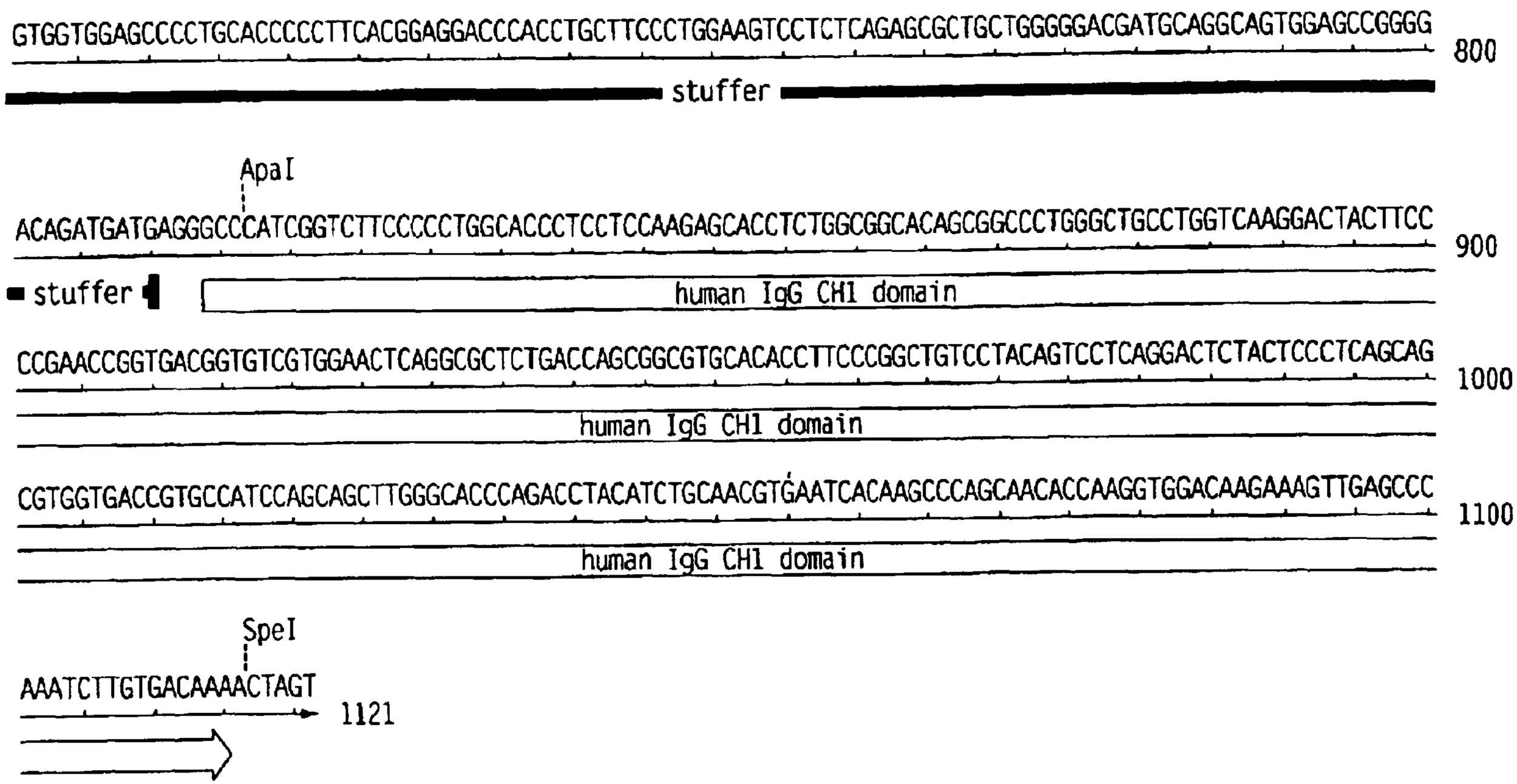
GTGGTGGAGCCCCTGCACCCCCTTCACGGAGGACCCACCTGCTTCCCTGGAAGTCCTCTCAGAGCGCTGCTGGGGGACGATGCAGGCAGTGGAGCCGGGG
800
stuffer
ApaI
ACAGATGATGAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGCGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCC
900
stuffer
human IgG CH1 domain
CCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAG
1000
human IgG CH1 domain
CGTGGTGACCGTGCCATCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCC
1100
human IgG CH1 domain
SpeI
AAATCTTGTGACAAAACTAGT
1121

FIG. 12C (Cont.)

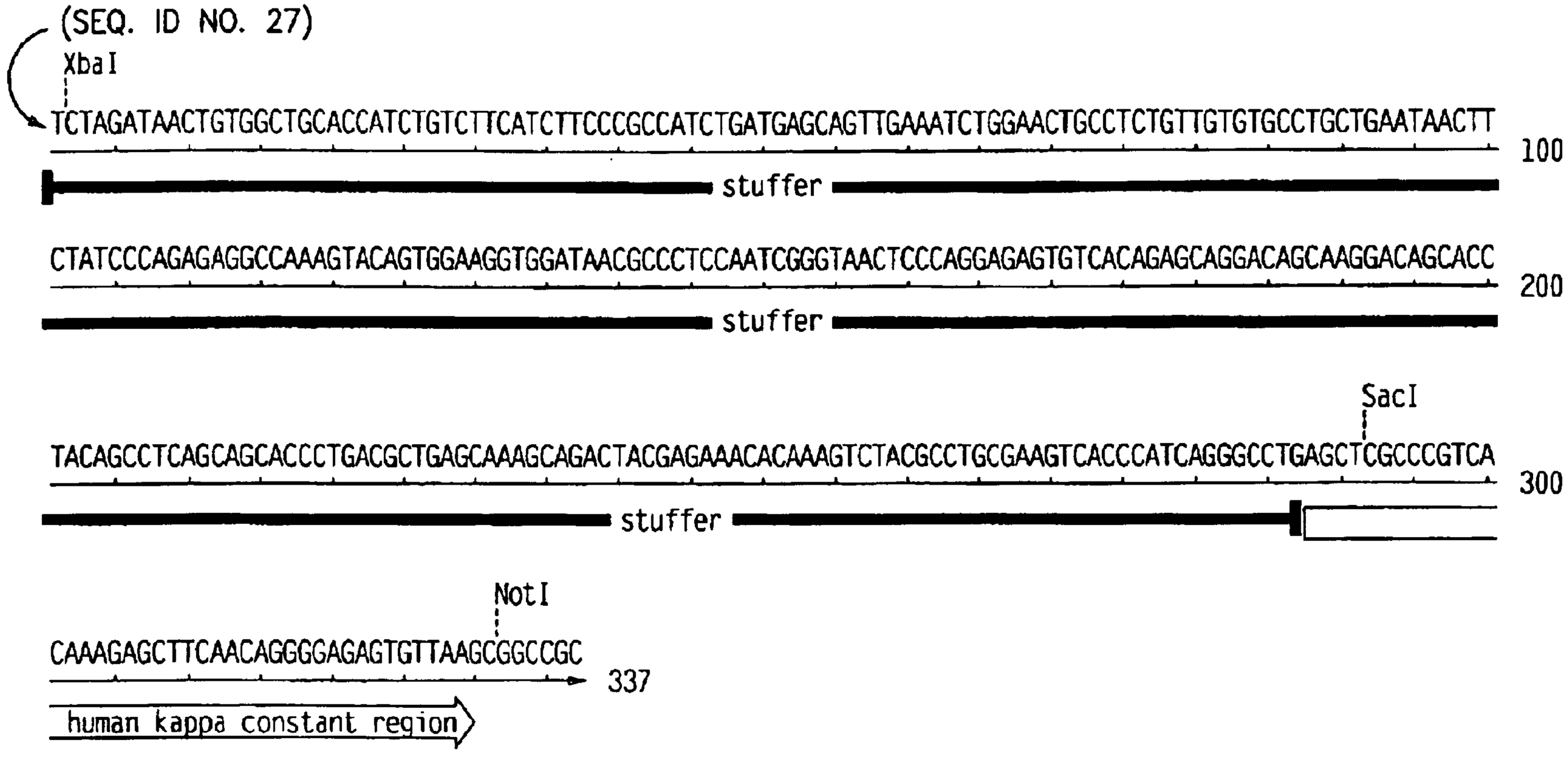
(SEQ. ID NO. 27)
XbaI
TCTAGATAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTT
100
stuffer
CTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACC
200
stuffer
SacI
TACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
300
stuffer
NotI
CAAAGAGCTTCAACAGGGGAGAGTGTTAAGCGGCCGC
337
human kappa constant region

FIG. 12D

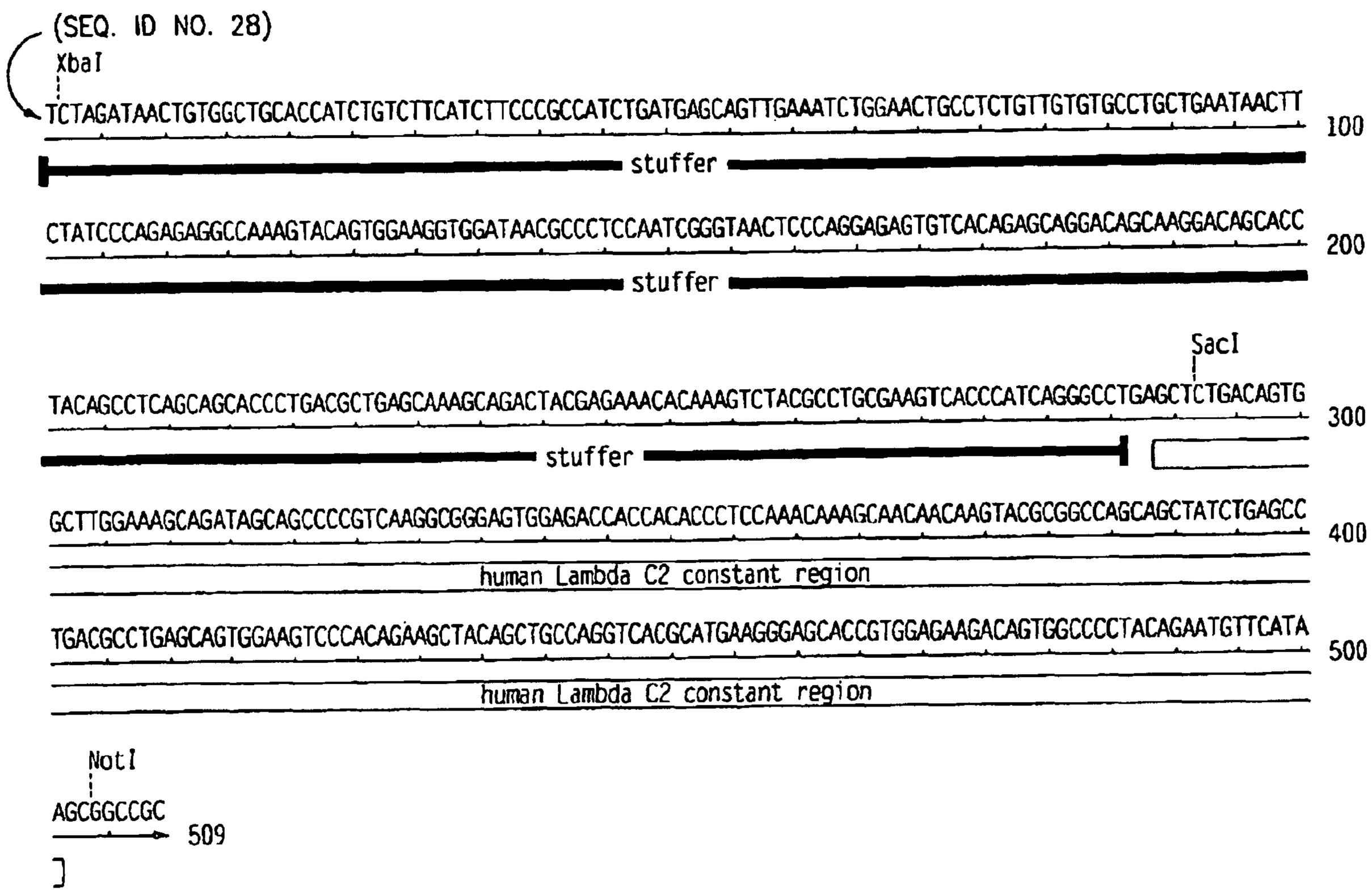
(SEQ. ID NO. 28)
XbaI
TCTAGATAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTT
100
stuffer
CTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACC
200
stuffer
SacI
TACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCTGACAGTG
300
stuffer
GCTTGGAAAGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCC
400
human Lambda C2 constant region
TGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTCATA
500
human Lambda C2 constant region
NotI
AGCGGCCGC
509

FIG. 12E

(SEQ. ID NO. 29)
XhoI
CTCGAGCTGATGAGCCATGGAAGCTGTGTCGCCTGCACCAGGCTCCCACGGCTCGTGGTGCGGTGCGCTTCTGGTGTTCGCTGCCTACAGCCGACACGTC 100
stuffer
GAGCTTCGTGCCCCTAGAGTTGCGCGTCACAGCAGCCTCCGGCGCTCCGCGATATCACCGTGTCATCCACATCAATGAAGTAGTGCTCCTAGACGCCCCC 200
stuffer
GTGGGGCTGGTGGCGCGGTTGGCTGACGAGAGCGGCCACGTAGTGTTGCGCTGGCTCCCGCCGCCTGAGACACCCATGACGTCTCACATCCGCTACGAGG 300
stuffer
TGGACGTCTCGGCCGGCAACGGCGCAGGGAGCGTACAGAGGGTGGAGATCCTGGAGGGCCGCACCGAGTGTGTGCTGAGCAACCTGCGGGGCCGGACGCG 400
stuffer
CTACACCTTCGCCGTCCGCGCGCGTATGGCTGAGCCGAGCTTCGGCGGCTTCTGGAGCGCCTGGTCGGAGCCTGTGTCGCTGCTGACGCCTAGCGACCTG 500
stuffer
GACCCCCTCATCCTGACGCTCTCCCTCATCCTCGTGGTCATCCTGGTGCTGCTGACCGTGCTCGCGCTGCTCTCCCACCGCCGGGCTCTGAAGCAGAAGA 600
stuffer
TCTGGCCTGGCATCCCGAGCCCAGAGAGCGAGTTTGAAGGCCTCTTCACCACCCACAAGGGTAACTTCCAGCTGTGGCTGTACCAGAATGATGGCTGCCT 700
stuffer

FIG. 12F

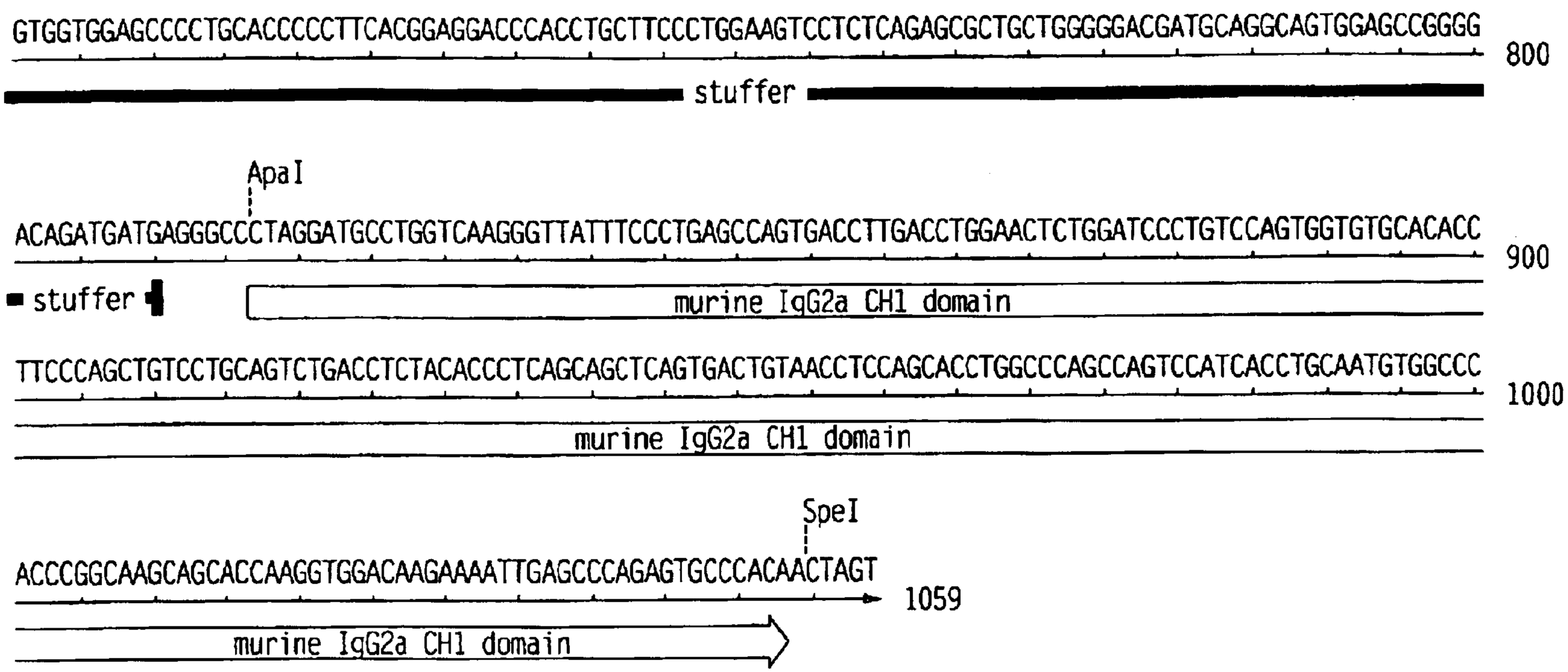
GTGGTGGAGCCCCTGCACCCCCTTCACGGAGGACCCACCTGCTTCCCTGGAAGTCCTCTCAGAGCGCTGCTGGGGGACGATGCAGGCAGTGGAGCCGGGG
800
stuffer
ApaI
ACAGATGATGAGGGCCCTAGGATGCCTGGTCAAGGGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACTCTGGATCCCTGTCCAGTGGTGTGCACACC
900
stuffer
murine IgG2a CH1 domain
TTCCCAGCTGTCCTGCAGTCTGACCTCTACACCCTCAGCAGCTCAGTGACTGTAACCTCCAGCACCTGGCCCAGCCAGTCCATCACCTGCAATGTGGCCC
1000
murine IgG2a CH1 domain
SpeI
ACCCGGCAAGCAGCACCAAGGTGGACAAGAAAATTGAGCCCAGAGTGCCCACAACTAGT
1059
murine IgG2a CH1 domain

FIG. 12F (Cont.)

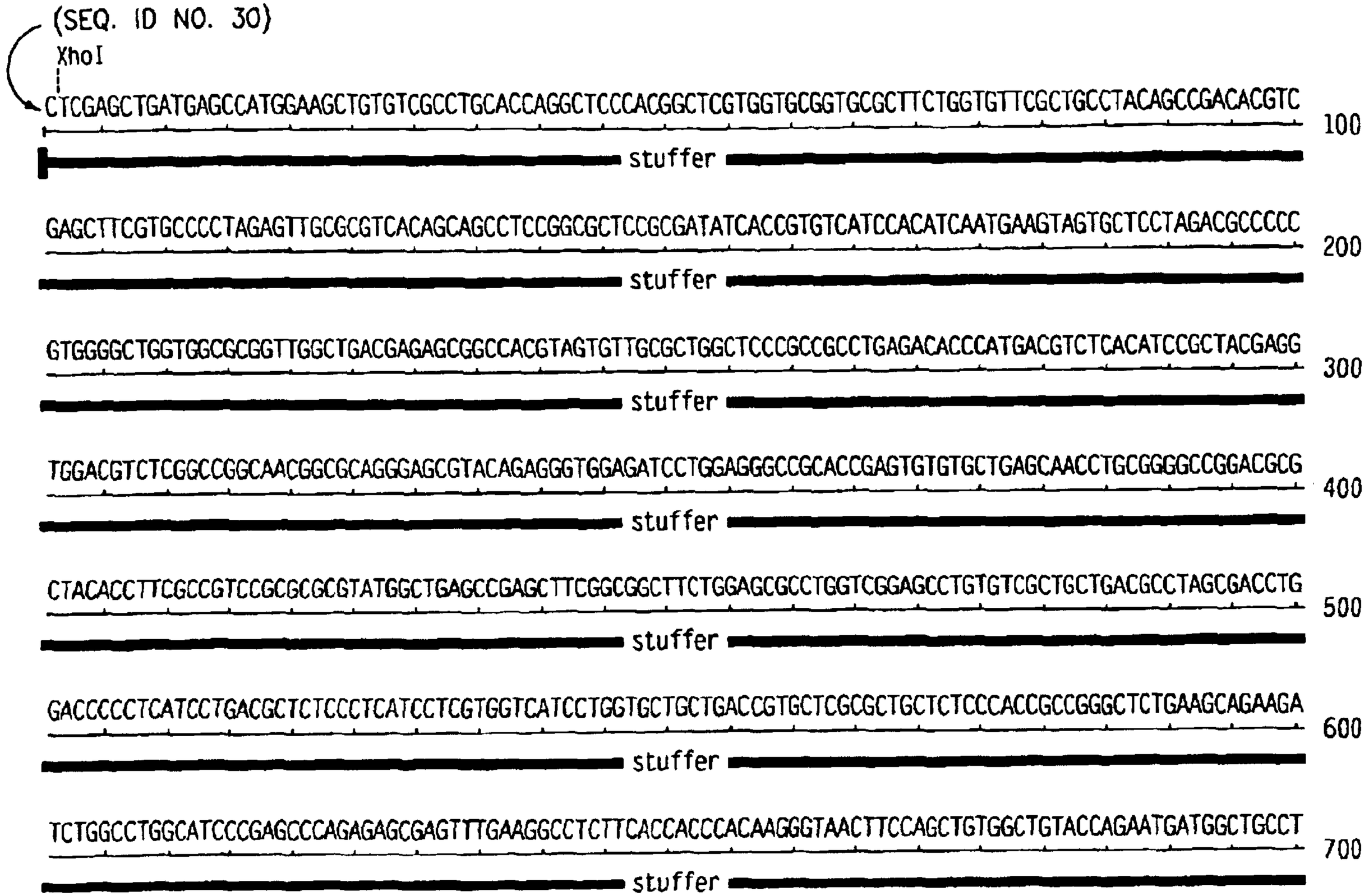
(SEQ. ID NO. 30)
XhoI
CTCGAGCTGATGAGCCATGGAAGCTGTGTCGCCTGCACCAGGCTCCCACGGCTCGTGGTGCGGTGCGCTTCTGGTGTTCGCTGCCTACAGCCGACACGTC
100
stuffer
GAGCTTCGTGCCCCTAGAGTTGCGCGTCACAGCAGCCTCCGGCGCTCCGCGATATCACCGTGTCATCCACATCAATGAAGTAGTGCTCCTAGACGCCCCC
200
stuffer
GTGGGGCTGGTGGCGCGGTTGGCTGACGAGAGCGGCCACGTAGTGTTGCGCTGGCTCCCGCCGCCTGAGACACCCATGACGTCTCACATCCGCTACGAGG
300
stuffer
TGGACGTCTCGGCCGGCAACGGCGCAGGGAGCGTACAGAGGGTGGAGATCCTGGAGGGCCGCACCGAGTGTGTGCTGAGCAACCTGCGGGGCCGGACGCG
400
stuffer
CTACACCTTCGCCGTCCGCGCGCGTATGGCTGAGCCGAGCTTCGGCGGCTTCTGGAGCGCCTGGTCGGAGCCTGTGTCGCTGCTGACGCCTAGCGACCTG
500
stuffer
GACCCCCTCATCCTGACGCTCTCCCTCATCCTCGTGGTCATCCTGGTGCTGCTGACCGTGCTCGCGCTGCTCTCCCACCGCCGGGCTCTGAAGCAGAAGA
600
stuffer
TCTGGCCTGGCATCCCGAGCCCAGAGAGCGAGTTTGAAGGCCTCTTCACCACCCACAAGGGTAACTTCCAGCTGTGGCTGTACCAGAATGATGGCTGCCT
700
stuffer

FIG. 12G

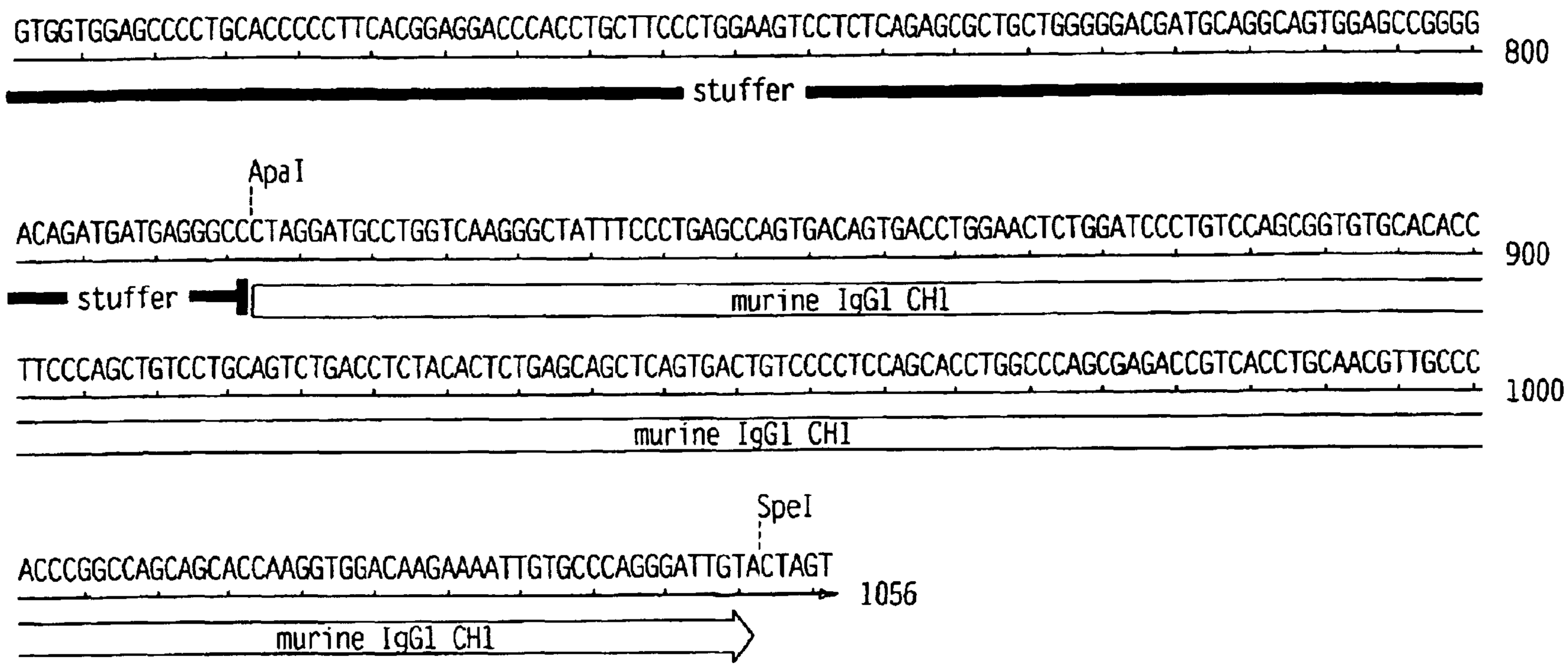
GTGGTGGAGCCCCTGCACCCCCTTCACGGAGGACCCACCTGCTTCCCTGGAAGTCCTCTCAGAGCGCTGCTGGGGGACGATGCAGGCAGTGGAGCCGGGG
800
stuffer
ApaI
ACAGATGATGAGGGCCCTAGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACC
900
stuffer
murine IgG1 CH1
TTCCCAGCTGTCCTGCAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCC
1000
murine IgG1 CH1
SpeI
ACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTACTAGT
1056
murine IgG1 CH1

FIG. 12G (Cont.)

(SEQ. ID NO. 31)

GTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAAT 100

GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCAC 200

beta lactamase

CCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTT 300

beta lactamase

TTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCG 400

beta lactamase

CCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCC 500

beta lactamase

ATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAA 600

beta lactamase

CTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACT 700

beta lactamase

FIG. 13A


ATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCG
800
beta lactamase
GCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAG
900
beta lactamase
TTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCA
1000
beta lactamase
AGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCT
1100
TAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAA
1200
col E1
CAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAA
1300
col E1
TACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCT
1400
col E1
GCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGC
1500
col E1

FIG. 13A (Cont.)

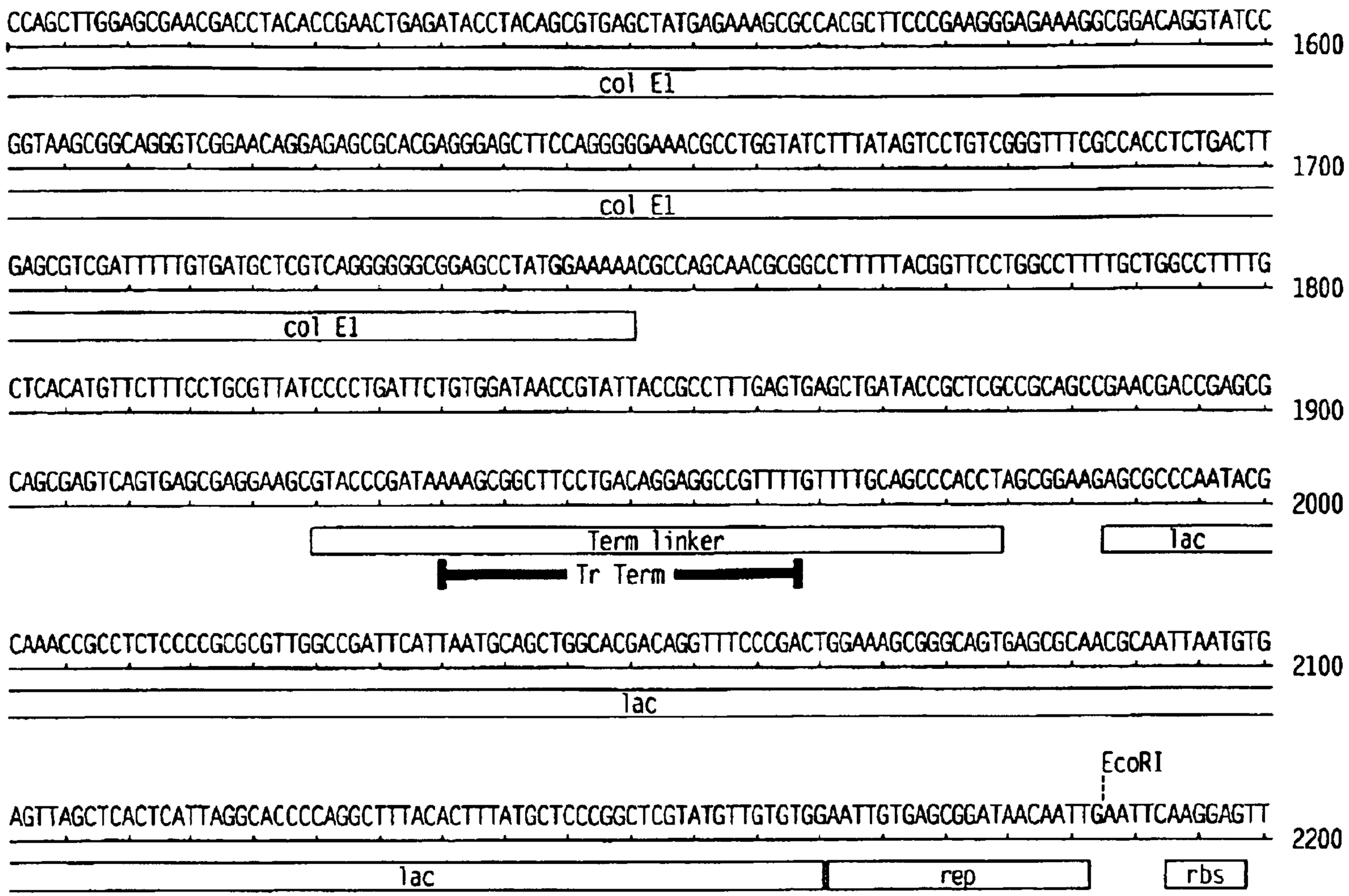
CCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCC
1600
col E1
GGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTT
1700
col E1
GAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTG
1800
col E1
CTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCG
1900
CAGCGAGTCAGTGAGCGAGGAAGCGTACCCGATAAAAGCGGCTTCCTGACAGGAGGCCGTTTTGTTTTGCAGCCCACCTAGCGGAAGAGCGCCCAATACG
2000
Term linker
lac
Tr Term
CAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTG
2100
lac
EcoRI
AGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTCCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTGAATTCAAGGAGTT
2200
lac
rep
rbs

FIG. 13B

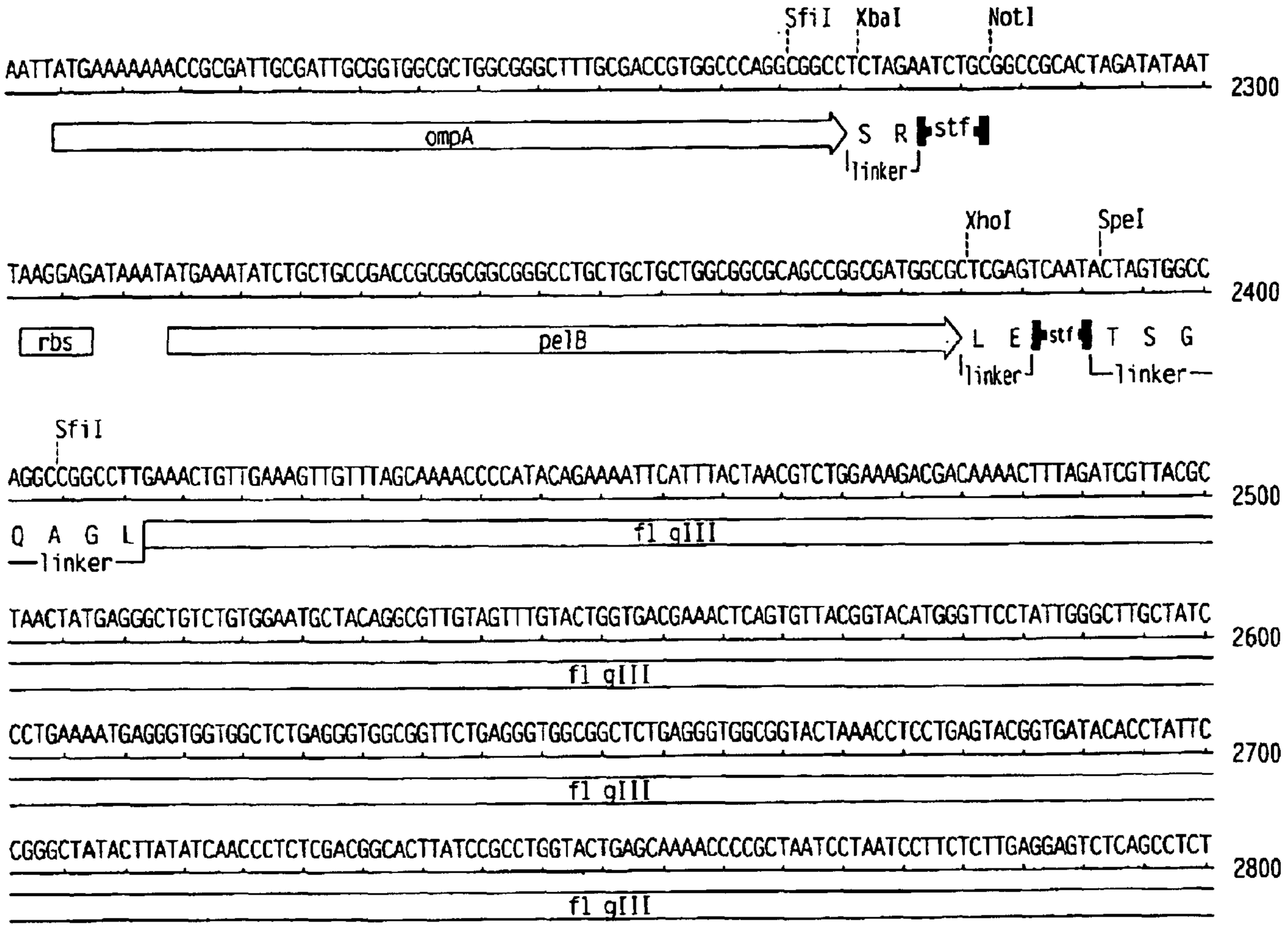
SfiI
XbaI
NotI
AATTATGAAAAAAACCGCGATTGCGATTGCGGTGGCGCTGGCGGGCTTTGCGACCGTGGCCCAGGCGGCCTCTAGAATCTGCGGCCGCACTAGATATAAT
2300
ompA
S R stf
linker
XhoI
SpeI
TAAGGAGATAAATATGAAATATCTGCTGCCGACCGCGGCGGCGGGCCTGCTGCTGCTGGCGGCGCAGCCGGCGATGGCGCTCGAGTCAATACTAGTGGCC
2400
rbs
pelB
L E stf T S G
linker
linker
SfiI
AGGCCGGCCTTGAAACTGTTGAAAGTTGTTTAGCAAAACCCCATACAGAAAATTCATTTACTAACGTCTGGAAAGACGACAAAACTTTAGATCGTTACGC
2500
Q A G L
linker
fl gIII
TAACTATGAGGGCTGTCTGTGGAATGCTACAGGCGTTGTAGTTTGTACTGGTGACGAAACTCAGTGTTACGGTACATGGGTTCCTATTGGGCTTGCTATC
2600
fl gIII
CCTGAAAATGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGTGGCGGTACTAAACCTCCTGAGTACGGTGATACACCTATTC
2700
fl gIII
CGGGCTATACTTATATCAACCCTCTCGACGGCACTTATCCGCCTGGTACTGAGCAAAACCCCGCTAATCCTAATCCTTCTCTTGAGGAGTCTCAGCCTCT
2800
fl gIII

FIG. 13B (Cont.)

TAATACTTTCATGTTTCAGAATAATAGGTTCCGAAATAGGCAGGGGGCATTAACTGTTTATACGGGCACTGTTACTCAAGGCACTGACCCCGTTAAAACT 2900

f1 gIII

TATTACCAGTACACTCCTGTATCATCAAAAGCCATGTATGACGCTTACTGGAACGGTAAATTCAGAGACTGCGCTTTCCATTCTGGCTTTAATGAGGATC 3000

f1 gIII

CATTCGTTTGTGAATATCAAGGCCAATCGTCTGACCTGCCTCAACCTCCTGTTAATGCTGGCGGCGGCTCTGGTGGTGGTTCTGGTGGCGGCTCTGCGGG 3100

f1 gIII

TGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGTGGCGGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCA 3200

f1 gIII

AACGCTAATAAGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTCTGACGCTAAAGGCAAACTTGATTCTGTCGCTACTGATTACGGTGCTG 3300

f1 gIII

CTATCGACGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGTGATTTTGCTGGCTCTAATTCCCAAATGGCTCAAGTCGGTGA 3400

f1 gIII

CGGTGATAATTCACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGAATGTCGCCCTTTTGTCTTTGGCGCTGGTAAACCA 3500

f1 gIII

TATGAATTTTCTATTGATTGTGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCACCTTTATGTATGTATTTTCGACGTTTG 3600

f1 gIII

FIG. 13C

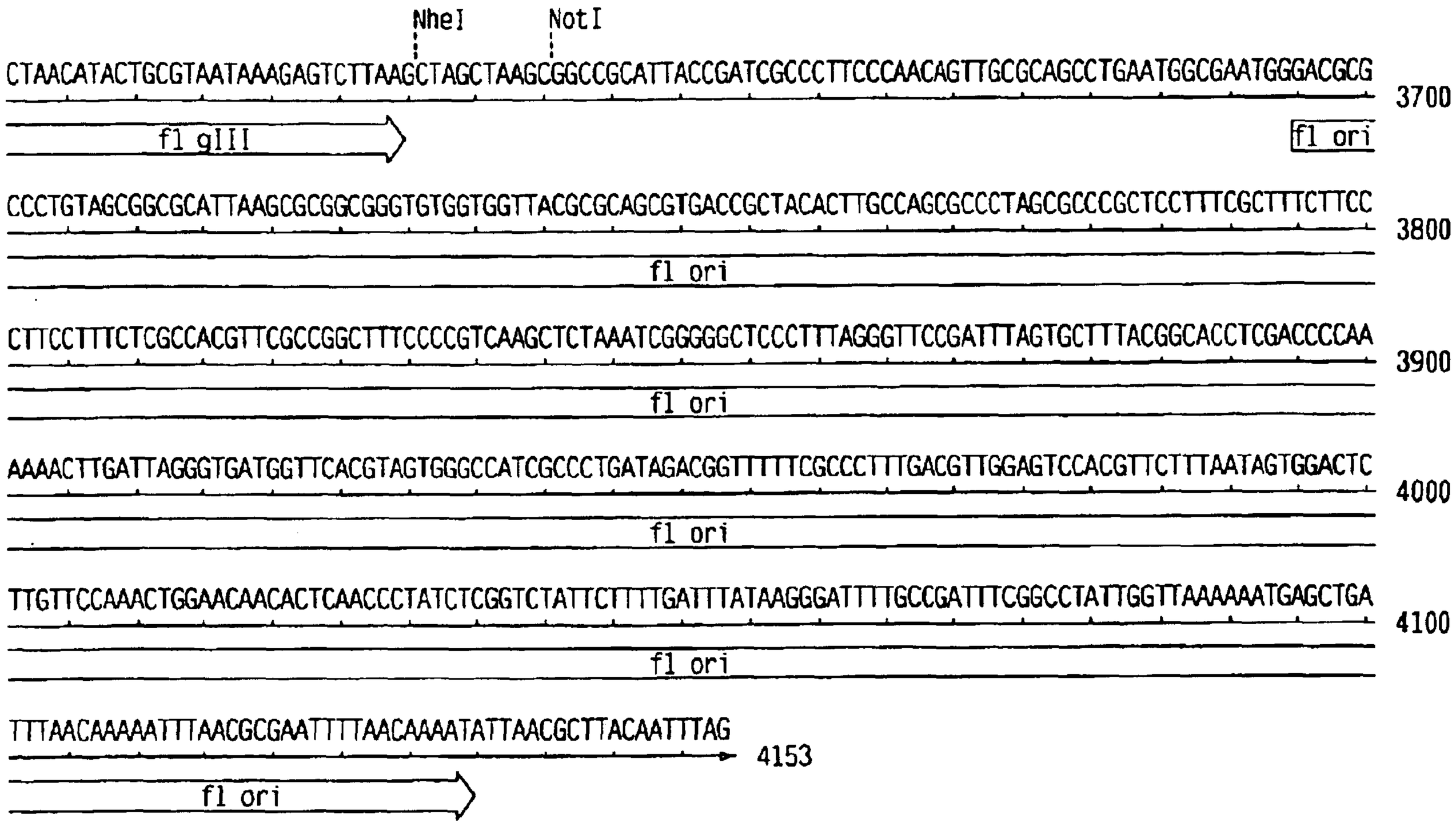
NheI
NotI
CTAACATACTGCGTAATAAAGAGTCTTAAGCTAGCTAAGCGGCCGCATTACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCG
3700
f1 gIII
f1 ori
CCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCC
3800
f1 ori
CTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAA
3900
f1 ori
AAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTC
4000
f1 ori
TTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGA
4100
f1 ori
TTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTAG
4153
f1 ori

FIG. 13C (Cont.)

PHAGEMID VECTORS

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

This application claims priority under 35 USC 119(e) to provisional application 60/287,355, filed Apr. 27, 2001.

BACKGROUND

1. Technical Field

This disclosure relates to cloning vectors. More specifically, phagemid vectors useful in the cloning and expression of foreign genetic information are disclosed.

2. Background of Related Art

Plasmids are extrachromosomal genetic elements capable of autonomous replication within their hosts. Bacterial plasmids range in size from 1 Kb to 200 Kb or more and encode a variety of useful properties. Plasmid encoded traits include resistance to antibiotics, production of antibiotics, degradation of complex organic molecules, production of bacteriocins, such as colicins, production of enterotoxins, and production of DNA restriction and modification enzymes.

Although plasmids have been studied for a number of years in their own right, particularly in terms of their replication, transmissibility, structure and evolution, with the advent of genetic engineering technology the focus of plasmid research has turned to the use of plasmids as vectors for the cloning and expression of foreign genetic information. In its application as a vector, the plasmid should possess one or more of the following properties. The plasmid DNA should be relatively small but capable of having relatively large amounts of foreign DNA incorporated into it. The size of the DNA insert is of concern in vectors based on bacteriophages where packing the nucleic acid into the phage particles can determine an upper limit. The plasmid should be under relaxed replication control. That is, where the replication of the plasmid molecule is not strictly coupled to the replication of the host DNA (stringent control), thereby resulting in multiple copies of plasmid DNA per host cell. The plasmid should express one or more selectable markers, such as the drug resistance markers, mentioned above, to permit the identification of host cells which contain the plasmid and also to provide a positive selection pressure for the maintenance of the plasmid in the host cell. Finally the plasmid should contain a single restriction site for one or more endonucleases in a region of plasmid which is not essential for plasmid replication. A vector as described above is useful, for example, for cloning genetic information, by which is meant integrating a segment of foreign DNA into the vector and reproducing identical copies of that information by virtue of the replication of the plasmid DNA.

The next step in the evolution of vector technology was the construction of so-called expression vectors. These vectors are characterized by their ability not only to replicate the inserted foreign genetic information but also to promote the transcription of the genetic information into mRNA and its subsequent translation into protein. This expression requires a variety of regulatory genetic sequences including but not necessarily limited to promoters, operators, transcription terminators, ribosomal binding sites and protein synthesis initiation and termination codons. These expression elements can be provided with the foreign DNA segment as parts thereof or can be integrated within the vector in a

region adjacent to a restriction site so that when a foreign DNA segment is introduced into the vector it falls under the control of those elements to which it is now chemically joined.

Filamentous bacteriophage consist of a circular, single-stranded DNA molecule surrounded by a cylinder of coat proteins. There are about 2,700 molecules of the major coat proteins pVIII that envelope the phage. At one end of the phage particle, there are approximately five copies of each of gene III and VI proteins (pIII and pVI) that are involved in host cell binding and in the termination of the assembly process. The other end contains five copies of each of pVII and pIX that are required for the initiation of assembly and for maintenance of virion stability. In recent years, vectors have been developed and utilized for the display of foreign peptides and proteins on the surface of filamentous phage or phagemid particles.

The display of peptides and proteins on the surface of phage or phagemid particles represents a powerful methodology for selection of rare members in a complex library and for carrying out molecular evolution in the laboratory. The ability to construct libraries of enormous molecular diversity and to select for molecules with predetermined properties has made this technology applicable to a wide range of problems. A few of the many applications of such technology are: i) phage display of natural peptides including, mapping epitopes of monoclonal and polyclonal antibodies and generating immunogens; ii) phage display of random peptides, including mapping epitopes of monoclonal and polyclonal antibodies, identifying peptide ligands, and mapping substrate sites for proteases and kinases; and iii) phage display of protein and protein domains, including directed evolution of proteins, isolation of antibodies and cDNA expression screening.

Vectors have been developed which incorporate DNA from plasmids and bacteriophage. These phagemid vectors are derived by modifications of a plasmid genome containing an origin of replication from a bacteriophage, (e.g. f1, M13, fd) as well as the plasmid origin of replication. Phagemids are useful for the expression of foreign genetic information.

One known phagemid vector is **[**pBluescript**]** *PBLUESCRIPT*™ II KS+ (pBS II KS+) (Stratagene, La Jolla, Calif.), which is a useful starting point for the construction of the present vector because of its small size and the fact that it contains the colE1 plasmid origin of replication and the phage f1 origin of replication in the desired orientation. The plasmid also carries an ampicillin resistance gene.

Vectors which due to their structures provide enhanced functionality would be desirable.

SUMMARY

Novel plasmid vectors capable of replication and expression of foreign genetic information in bacteria, such as, for example, cyanobacterium and E. coli are described herein. These new vectors contain a specific sequence of features after the ColE1 origin but before the f1 origin. Specifically, the present phagemid vector contains, after the ColE1 origin but before the f1 origin, a bacterial transcription terminator, a promoter, a first ribosomal binding site, a first leader sequence and a first cloning region, a second ribosomal binding site, a second leader sequence and a second cloning region. The second cloning region is adapted to receive a gene encoding a polypeptide to be displayed and a nucleotide sequence encoding at least a functional domain of a display protein.

The vectors described herein are constructed through a series of steps which convert a starting vector through a series of intermediate plasmids to the present novel vector which can be used for display of antibody libraries.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** schematically illustrates the structure of pBS II KS+, a useful starting vector for making the novel vectors described herein;

FIG. **2** is a flow chart illustrating the method of making the novel vectors described herein;

FIG. **3** schematically illustrates the digestion of the starting vector and insertion of the promoter;

FIGS. **4**A–C show the sequence (Seq. ID No. 19) of intermediate vector p110-81.6;

FIG. **5** schematically illustrates the insertion of the terminator;

FIGS. **6**A–C show the sequence (Seq. ID No. 20) of intermediate vector p131-03.7;

FIG. **7** schematically illustrates the insertion of multiple cloning sites;

FIGS. **8**A–C show the sequence (Seq. ID No. 21) of intermediate vector p131-39.1;

FIG. **9** schematically illustrates the insertion of the nucleotide sequence encoding the display protein and the two transcriptional control cassettes;

FIG. **10** is a map of plasmid pAX131; and

FIGS. **11**A–D show the nucleic acid sequence (Seq. ID No. 18) of plasmid pAX131, including the domains corresponding to particular genes.

FIGS. **12**A–G show the nucleic acid sequences of illustrative stuffer sequences.

FIGS. **13**A–C show the nucleic acid sequence of plasmid pAX131 Xba/Not.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present novel phagemid vectors are useful for display of polypeptides such as, for example, antibody libraries. The vectors described herein can be prepared using any commercially available vector containing a ColE1 and an f1 origin of replication as the starting material. Such starting materials are known and are commercially available. One suitable starting material is the vector pBS II KS+ which is commercially available from Stratagene Corp., La Jolla, Calif. (Sec FIG. **1**).

FIG. **2** is a flow-chart showing one embodiment of the steps involved in converting a starting vector into one of the present novel vectors. Those skilled in the art will readily envision other schemes for preparing the present vectors. Accordingly, the present disclosure is not limited to the sequence of steps shown in FIG. **2**.

In the first step, the starting vector is digested with restriction enzymes to remove a substantial portion of the vector between the ColE1 origin and the f1 origin of replication. Typically, the portion to be removed from the starting vector includes multiple cloning sites. Depending on the particular restriction sites present in the starting vector, suitable methods for digesting the starting vector are known to and readily selected by those skilled in the art.

Next, a promoter is inserted downstream of the ColE1 origin of the digested starting vector. Any promoter recognized by a host cell can be employed. Suitable promoters include, but are not limited to, ara, lac and trc promoters. The promoter drives expression of other sequences inserted into the vector, such as, for example expression of polypeptides. In particularly useful embodiments, a promoter sequence generated from the starting vector is employed as the promoter inserted downstream of the ColE1 origin as described in more detail below.

In the next step, a bacterial transcription terminator is inserted downstream of the ColE1 origin, and upstream of the promoter. Any terminator recognized by a host cell can be employed. Suitable terminators include, but are not limited to, the t_{HP} terminator, the bgIG terminator, and the crp terminator. It should be noted that bioinformatics analysis has allowed the identification of over 100 rho-independent transcription terminators in the E. coli genome, all of which should be suitable for this purpose (Ermolaeva, et al, J. Mol Biol 301:27–33 (2000)).

In the next step, multiple restriction sites are inserted downstream of the promoter. The restriction site can be any known restriction site. Suitable restriction sites for insertion include, but are not limited to Nhe I, Hind III, Nco I, Xma I, Bgl II, Bst I, Pvu I, etc. The number of restriction sites inserted is not critical, provided a sufficient number of restriction sites are inserted to allow completion of the balance of the steps needed to create the present novel vectors. Thus as few as 2 to as many as 10 or more restriction sites can be inserted in this step. It should be understood that if one or more of the restriction sites selected for insertion is present in the starting vector, it may be desirable to remove or disable the native restriction site to avoid unwanted digestion during further processing. The restriction site can be inserted using any technique known to those skilled in the art. A particularly preferred combination of restriction sites inserted in this step is Not I, Sfi I, Spe I, Xho I, Xba I and EcoR I.

The next step involves inserting a nucleotide sequence encoding a product that enables display of a polypeptide on the surface of a phagemid particle. The product encoded can thus be considered at least a functional domain of a display protein. The display protein can be any natural or synthetic polypeptide to which a polypeptide to be displayed can be fused and which can present the polypeptide to be displayed for screening processes. Suitable display polypeptides include proteins that can be incorporated into the coat of a phage particle. As those skilled in the art will appreciate, filamentous bacteriophage consist of a circular, single-stranded DNA molecule a surrounded by a cylinder of coat proteins. There are about 2,700 molecules of the major coat protein pVIII that encapsidate the phage. At one end of the phage particle, there are approximately five copies each of gene III and VI proteins (pIII and pVI) that are involved in host-cell binding and in the termination of the assembly process. The other end contains five copies each of pVII and pIX that are required for the initiation of assembly and for maintenance of virion stability. A nucleotide sequence encoding any of these coat proteins can be employed in making the novel vectors herein. Particularly preferred are nucleotide sequences encoding at least a functional domain of pIII. The nucleotide sequence encoding at least a functional domain of pill can be natural or synthetic. The nucleotide sequence inserted can encode a truncated pIII provided the display function of the protein is maintained. An example of a synthetic or artificial coat protein useful herein is that disclosed in Weiss et al., Mol Biol., 300(1), 213–219 (2000), the disclosure of which is incorporated herein by reference.

In the next step, two transcriptional control cassettes are inserted, an upstream transcriptional control cassette and a

downstream transcriptional control cassette. Each of the transcriptional control cassettes include a ribosomal binding site, a leader sequence and a cloning site for receiving a gene encoding a polypeptide to be expressed. Any known ribosomal binding site (RBS) and leader sequence recognized by the host cell can be employed. Preferably, the RBS and leader sequence employed is optimized for expression in E. coli. The cloning site is a region of the nucleic acid between two restriction sites, typically with a nonessential region of nucleotide sequence (commonly referred to as a "stuffer" sequence) positioned therebetween. Alternatively, the stuffer sequence may contain a non-essential region and a portion of an antibody constant domain. Suitable stuffer sequences include, for example, those shown in FIGS. **12**A–G.

The downstream transcriptional control cassette is inserted adjacent to the nucleotide sequence encoding at least the functional domain of the display protein. In this manner, a fusion protein will be expressed when a gene encoding a polypeptide to be displayed is inserted at the cloning site of the downstream transcriptional control cassette. As those skilled in the art will appreciate, a suppressible stop codon could be positioned between the gene encoding the polypeptide to be displayed and the nucleotide sequence encoding at least a functional domain of the display protein such that fusion display is obtained in a suppressing host (as long as the gene is inserted in-frame) and a secreted protein without the display protein is obtained in a non-suppressing host.

The upstream transcriptional control cassette is inserted upstream of the downstream transcriptional control cassette. The upstream transcriptional control cassette provides a second cloning region for receiving a second gene encoding a polypeptide that can dimerize with the polypeptide to be displayed. For example, where the vector expresses a heavy chain Fd fused to a display protein, the second gene preferably encodes an antibody light chain. As with the cloning site of the downstream transcriptional control cassette, the cloning site of the upstream transcriptional control cassette is a region of the vector between two restriction sites, typically with a stuffer positioned therebetween. It should of course be understood that where a polypeptide other than an antibody is to be displayed (such as, for example, where monomeric display of a single polypeptide or protein is intended) a second gene need not be cloned into the vector at the cloning site of the upstream transcriptional control cassette. In such cases the second cloning site can simply remain unused. As those skilled in the art will also appreciate, where a single chain antibody is encoded by the gene inserted at the cloning site of the downstream transcriptional control cassette, there is no need to insert a second gene into the vector at the cloning site of the upstream transcriptional control cassette.

Thus, the phagmid vector produced by the process illustrated in FIG. **2** will contain, after the ColE1 origin but before the f1 origin, a terminator, a promoter, a first ribosomal binding site, a first leader sequence and a first cloning region, a second ribosomal binding site, a second leader sequence and, a second cloning region for receiving a gene encoding a polypeptide to be displayed and a nucleotide sequence encoding at least a functional domain of a display protein.

The present vectors also include a selectable marker. Either an ampicillin resistant or a CAT resistant vector can be produced in accordance with the present disclosure. The ampicillin or CAT resistance can be provided by simply choosing a starting vector having the desired resistance. Alternatively, if the starting vector is ampicillin resistant to produce a CAT resistant vector, the ampicillin resistant gene is removed and replaced with the chloramphenicol transferase gene. Techniques for providing either ampicillin or CAT resistance in the present vectors will be readily apparent to those skilled in the art. Other suitable selectable markers include, but are not limited to, tetracycline or kanamycin resistance.

The vectors described herein can be transformed into a host cell using known techniques (e.g., electroporation) and amplified. The vectors described herein can also be digested and have a first gene and optionally a second gene ligated therein in accordance with this disclosure. The vector so engineered can be transformed into a host cell using known techniques and amplified or to effect expression of polypeptides and/or proteins encoded thereby to produce phage particles displaying single polypeptides or dimeric species. Those skilled in the art will readily envision other uses for the novel vectors described herein.

The following examples illustrate the present invention without limiting its scope. The steps involved in constructing the vectors described herein are discussed in detail in the Examples. Those skilled in the art possess knowledge of suitable techniques to accomplish the steps described below without the need for undue experimentation, such techniques being well known to those skilled in the art.

EXAMPLE 1

This example illustrates methods and compositions for the construction of one embodiment of a phagemid vector according to the present disclosure. The starting phagemid selected for construction was pBS II KS+ which contains an ampicillin resistant gene which results in a final vector, pAX131, which is ampicillin resistant.

Digestion of Starting Vector and Insertion of Promoter

The commercially available vector pBS II KS+ (Stratagene, LaJolla, Calif.) was digested with Pvu I and Sap I to generate a 2424 bp pBS II KS+ fragment which lacks the bases at positions 500 to 1037 corresponding to the multiple cloning region. The resulting fragment contains the Ampicillin resistant gene (AmpR), phage f1 origin, and the Col E1origin. (See FIG. **3**.) Next, two mutagenic primers were used with the pBS II KS+ fragment in a PCR reaction followed by digestion with EcoR I and Sap I to generate a 209 bp fragment containing the lac promoter. The primers used were as follows:

5' AAC CGT ATT ACC GCC TTT GAG TG 3' (SEQ. ID. NO. 1):

and

5' CCT GAA TTC AAT TGT TAT CCG CTC ACA ATT CCA C 3' (SEQ. ID. NO. 2).

The 2424 bp fragment and the 209 bp fragment were combined in a three-way ligation reaction with two overlapping oligonucleotides which contain a Not I, EcoR I and Pvu I sites to form a first intermediate plasmid (designated p110-81.6). (See FIG. **3**.) The oligonucleotides used for this reaction were:

5' CGG TAA TGC GGC CGC TAC ATG 3' (SEQ. ID. NO. 3);

and

5' AAT TCA TGT AGC GGC CGC ATT ACC GAT 3' (SEQ. ID. NO. 4).

The resulting plasmid p110-81.6 was digested and sequenced in the altered region to identify a clone with the correct incorporation of the lac promoter, Pvu I, Sap I, EcoR,

and Not I sites. The sequencing of p110-81.6 revealed a nucleic acid change at position 875 within the lac promoter. The published sequence of pBS II KS+ had an adenine at position 875. However, sequencing of p110-81.6 and the original pBS II KS+ revealed a guanine at position 875. The sequence (Seq. ID No. 19) of intermediate plasmid p110-81.6 is shown in FIGS. **4**A–C.

Insertion of Terminator

A transcription termination sequence was inserted into the first intermediate plasmid (p110-81.6) upstream of the lac promoter at the Sap I site. (See FIG. **5**.)

Plasmid 110-81.6 was digested with Sap I to create an insertion point for the oligonucleotides which contained a t_{HP} terminator (Nohno et al., Molecular and General Genetics, Vol. 205, pages 260-269 (1986). The oligonucleotides used in this ligation were:

5' AGC GTA CCC GAT AAA AGC GGC TTC CTG ACA GGA GGC CGT TTT GTT TTG CAG CCC ACC T 3'; (SEQ. ID. No. 5);

and

5' GCT AGG TGG GCT GCA AAA CAA AAC GGC CTC CTG TCA GGA AGC CGC TTT TAT CGG GTA C 3' (SEQ. ID. NO. 6).

The resulting intermediate vector (designated p131-03.7) was digested and sequenced in the altered region to determine its identity. The sequence (Seq. ID No. 20) of intermediate vector p131-03.7 is shown in FIGS. **6**A–C.

Insertion of Multiple Restriction Sites

Oligonucleotides containing the Xba I, XhoI, SpeI and Sfi sites were then inserted into intermediate plasmid p131-03.7.(See FIG. **7**.)

Intermediate vector p131-03.7 was digested with EcoR I and Not I and then gel purified. Then overlapping oligonucleotides containing the Xba I, Xho I, Spe I and Sfi I sites were ligated into the p131-03.7 backbone. The oligonucleotides inserted were:

5' AAT TCA CAT CTA GAT ATC TCG AGT CAA TAC TAG TGG CCA GGC CGG CCA GC 3' (SEQ. ID. NO. 7);

and

5' GGC CGC TGG CCG GCC TGG CCA CTA GTA TTG ACT CGA GAT ATC TAG ATG TG 3' (SEQ. ID. NO. 8).

The resulting intermediate plasmid (designated p131-39.1) was sequenced and analyzed to determine its identity. The sequence (Seq. ID No. 21) of intermediate plasmid p131-39.1 is shown in FIGS. **8**A–C.

Construction of Nucleotide Sequence Encoding Display Protein

Single stranded DNA from phage f1 (ATCC #15766-B2) was used as a template for the cloning of gene III. (Sec FIG. **9**.)

The primers used were:

5' AGT GGC CAG GCC GGC CTT GAA ACT GTT GAA AGT TGT TTA GCA AA 3' (SEQ. ID. NO. 9)

which contains the Sfi I site, bases to maintain the coding frame and a portion of gene III; and

5 TCT GCG GCC GCT TAG CTA GCT TAA GAC TCT TTA TTA CGC AGT ATG TTA GCA 3' (SEQ. ID. NO. 10);

which contains the end of gene III in which an internal ribosome binding site ordinarily used for the next downstream gene has been removed by changing a silent third base position in the corresponding codon. This oligonucleotide also contains a stop codon, Nhe I site for potential use in removal of the fusion, a second stop codon for use with the fusion, and the Not I site for cloning. The PCR fragment was digested with Sfi I and Not I and inserted into p131-39.1 digested with Sfi I and Not I to create intermediate vector p131-44.2. The integrity of the gene III region and flanking sequences was confirmed by sequence analysis.

Creation of the Upstream Transcriptional Control Cassette

Plasmid 131-39.1 was utilized as a shuttle vector for cloning the oligonucleotides containing the ompA signal peptide coding sequence. The upstream transcriptional control cassette was generated within intermediate plasmid 131-39.1 by inserting a pair of oligonucleotides which contain EcoR I, the ompA signal peptide leader, followed by a Sac I site, a small stuffer region, and a ribosome binding site. (See FIG. **9**.) The oligonucleotides used were:

Eco Xba:

5' AAT TCA AGG AGT TAA TTA TGA AAA AAA CCG CGA TTG CGA TTG CGG TGG CGC TGG CGG GCT TTG CGA CCG TGG CCC AGG CGG CCG AGC TCA TCT T 3' (SEQ. ID. NO. 11);

and

Xba Eco:

5' CTA GAA GAT GAG CTC GGC CGC CTG GGC CAC GGT CGC AAA GCC CGC CAG CGC CAC CGC AAT CGC AAT CGC GGT TTT TTT CAT AAT TAA CTC CTT G 3' (SEQ. ID. NO. 12).

The RBS and leader sequences included in the upstream transcriptional control cassette are optimized for use in E. coli. These novel sequences are:

5' AAG GAG 3' (Seq. ID No.13)

for the RBS; and

5' ATG AAA AAA ACC GCG ATT GCG ATT GCG GTG GCG CTG GCG GGC TTT GCG ACC GTG GCC CAG GCG GCC 3' (Seq. ID No. 14)

for the ompA leader. The resulting plasmid was sequenced to confirm the identity of the insert and digested at the EcoRI and Xbal sites to generate a 94 bp fragment which is the upstream transcriptional control cassette.

Creation of the Downstream Transcriptional Control Cassette

Intermediate plasmid 131-39.1 was utilized as a shuttle vector for cloning the oligonucleotides containing the pelB signal peptide coding sequence. The downstream transcriptional control cassette was generated within intermediate plasmid 131-39.1 by inserting a pair of oligonucleotides containing the pelB signal peptide, Xba I, site, and a ribosome binding site. The oligonucleotides used were:

XbaXho:

5' CTA GAT ATA ATT AAG GAG ATA AAT ATG AAA TAT CTG CTG CCG ACC GCG GCG GCG GGC CTG CTG CTG CTG GCG GCG CAG CCG GCG ATG GCGC 3' (SEQ. ID. NO. 15);

and

XhoXba:

5' TCG AGC GCC ATC GCC GGC TGC GCC GCC AGC AGC AGC AGG CCC GCC GCC GCG GTC GGC AGC AGA TAT

TTC ATA TTT ATC TCC TTA ATT ATA T 3' (SEQ. ID. NO. 16).

The novel pelB leader sequence was optimized for use in E. coli and had the sequence

5' TAT GAA ATA TCT GCT GCC GAC CGC GGC GGC GGG CCT GCT GCT GCT GGC GGC GCA GCC GGC GAT GGC G 3' (Seq. ID No. 17).

The resulting plasmid was sequenced to confirm the identity of the insert and digested at the Xbal and XhoI sites to generate a 91 bp fragment which is the downstream transcriptional control cassette.

Construction of pAx131 Vector

The upstream transcriptional control cassette and the downstream transcriptional control cassette were combined with intermediate plasmid p131-44.2 digested with EcoRI and XhoI in a 3-way ligation reaction to produce pAX131 (See FIG. **9**). FIG. **10** is a map of the resulting pAX131 vector. The pAX131 was analyzed to determine its nucleic acid sequence (SEQ. ID. NO. 18) which is shown in FIGS. **11**A–D.

EXAMPLE 2

Insertion of an alternate upstream transcriptional control cassette

PAX131 vector was digested with Not I restriction enzyme. The resulting DNA overhangs were then filled in with Klenow fragment Polymerase to blunt end the DNA followed by ligation. This was performed to remove the existing Not I site. The Not I deleted PAX131 vector was digested with EcoR I/Xba I, and ligated with a duplexed oligo containing EcoR I and Spe I overhangs (Xba I, and Spe I have compatible ends).

Eco/Spe oligo:

5' AAT TCA AGG AGT TAA TTA TGA AAA AAA CCG CGA TTG CGA TTG CGG TGG CGC TGG CGG GCT TTG CGA CCG TGG CCC AGG CGG CCT CTA GAA TCT GCG GCC GCA 3' (SEQ. ID NO. 22)

Spe/Eco oligo:

5' CTA GTG CGG CCG CAG ATT CTA GAG GCC GCC TGG GCC ACG GTC GCA AAG CCC GCC AGC GCC ACC GCA ATC GCA ATC GCG GTT TTT TTC ATA ATT AAC TCC TTG 3' (SEQ. ID NO. 23)

The resulting vector (pAX131 Xba/Not) had Xba I, and Not I sites for cloning of a gene, such as light chains, rather than Sac I and Xba I. FIGS. **13**A–C show the nucleic acid sequence for vector (pAX131 Xba/Not.

It is contemplated that the present novel vectors can be used in connection with the production and screening of libraries made in accordance with conventional phage display technologies. Both natural and synthetic antibody repertoires have been generated as phage displayed libraries. Natural antibodies can be cloned from B-cell mRNA isolated from peripheral blood lymphocytes, bone marrow, spleen, or other lymphatic tissue of a human or non-human donor. Donors with an immune response to the antigen(s) of interest can be used to create immune antibody libraries. Alternatively, non-immune libraries may be generated from donors by isolating naive antibody B cell genes. PCR using antibody specific primers on the 18st strand cDNA allows the isolation of light chain and heavy chain antibody fragments which can then be cloned into the display vector.

Synthetic antibodies or antibody libraries can be made up in part or entirely with regions of synthetically derived sequence. Library diversity can be engineered within variable regions, particularly within CDRs, through the use of degenerate oligonucleotides. For example, a single Fab gene may be modified at the heavy chain CDR3 position to contain random nucleotide sequences. The random sequence can be introduced into the heavy chain gene using an oligonucleotide which contains the degenerate coding region in an overlap PCR approach. Alternatively, degenerate oligo cassettes can be cloned into restriction sites that flank the CDR(s) to create diversity. The resulting library generated by this or other approaches can then be cloned into a display vector in accordance with this disclosure.

Upon introduction of the display library into bacteria, phage particles will be generated that have antibody displayed on the surface. The resulting collection of phage-displayed antibodies can be selected for those with the ability to bind to the antigen of interest using techniques known to those skilled in the art. Antibodies identified by this system can be used therapeutically, as diagnostic reagents, or as research tools.

It is contemplated that single and double stranded versions of the vectors described herein are within the scope of the present invention. It is well within the purview of those skilled in the art to prepare either single or double stranded vectors having the features described herein.

It will be understood that various modifications may be made to the embodiments described herein. For example, as those skilled in the art will appreciate, a first gene encoding a fusion protein having an antibody light chain to be fused to and displayed by pVIII and a second gene encoding a heavy chain Fd can be inserted into the vector at the newly created restriction site to provide effective antibody display. Therefore, the above description should not be construed as limiting, but merely as exemplifications of preferred embodiments. Those skilled in the art will envision other modifications within the scope and spirit of the claims appended hereto.

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ctttgatctt ttctacgggg tctgacgctc agtggaacga aaactcacgt taagggattt 1620

tggtcatgag attatcaaaa aggatcttca cctagatcct tttaaattaa aaatgaagtt 1680

ttaaatcaat ctaaagtata tatgagtaaa cttggtctga cagttaccaa tgcttaatca 1740

gtgaggcacc tatctcagcg atctgtctat ttcgttcatc catagttgcc tgactccccg 1800

tcgtgtagat aactacgata cgggagggct taccatctgg ccccagtgct gcaatgatac 1860

cgcgagaccc acgctcaccg gctccagatt tatcagcaat aaaccagcca gccggaaggg 1920

ccgagcgcag aagtggtcct gcaactttat ccgcctccat ccagtctatt aattgttgcc 1980

gggaagctag agtaagtagt tcgccagtta atagtttgcg caacgttgtt gccattgcta 2040

caggcatcgt ggtgtcacgc tcgtcgtttg gtatggcttc attcagctcc ggttcccaac 2100

gatcaaggcg agttacatga tcccccatgt tgtgcaaaaa agcggttagc tccttcggtc 2160

ctccgatcgt tgtcagaagt aagttggccg cagtgttatc actcatggtt atggcagcac 2220

tgcataattc tcttactgtc atgccatccg taagatgctt ttctgtgact ggtgagtact 2280

caaccaagtc attctgagaa tagtgtatgc ggcgaccgag ttgctcttgc ccggcgtcaa 2340

tacgggataa taccgcgcca catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt 2400

cttcggggcg aaaactctca aggatcttac cgctgttgag atccagttcg atgtaaccca 2460

ctcgtgcacc caactgatct tcagcatctt ttactttcac cagcgtttct gggtgagcaa 2520

aaacaggaag gcaaaatgcc gcaaaaaagg gaataagggc gacacggaaa tgttgaatac 2580

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tcatactctt ccttttttcaa tattattgaa gcatttatca gggttattgt ctcatgagcg 2640

gatacatatt tgaatgtatt tagaaaaata aacaaatagg ggttccgcgc acatttcccc 2700

gaaaagtgcc ac 2712

<210> SEQ ID NO 21
<211> LENGTH: 2750
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: plasmid vector

<400> SEQUENCE: 21

gtggcacttt tcggggaaat gtgcgcggaa cccctatttg tttatttttc taaatacatt 60

caaatatgta tccgctcatg agacaataac cctgataaat gcttcaataa tattgaaaaa 120

ggaagagtat gagtattcaa catttccgtg tcgcccttat tccctttttt gcggcatttt 180

gccttcctgt ttttgctcac ccagaaacgc tggtgaaagt aaaagatgct gaagatcagt 240

tgggtgcacg agtgggttac atcgaactgg atctcaacag cggtaagatc cttgagagtt 300

ttcgccccga agaacgtttt ccaatgatga gcacttttaa agttctgcta tgtggcgcgg 360

tattatcccg tattgacgcc gggcaagagc aactcggtcg ccgcatacac tattctcaga 420

atgacttggt tgagtactca ccagtcacag aaaagcatct tacggatggc atgacagtaa 480

gagaattatg cagtgctgcc ataaccatga gtgataacac tgcggccaac ttacttctga 540

caacgatcgg aggaccgaag gagctaaccg ctttttttgca caacatgggg gatcatgtaa 600

ctcgccttga tcgttgggaa ccggagctga atgaagccat accaaacgac gagcgtgaca 660

ccacgatgcc tgtagcaatg gcaacaacgt tgcgcaaact attaactggc gaactactta 720

ctctagcttc ccggcaacaa ttaatagact ggatggaggc ggataaagtt gcaggaccac 780

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gtgggtctcg cggtatcatt gcagcactgg ggccagatgg taagccctcc cgtatcgtag 900

ttatctacac gacggggagt caggcaacta tggatgaacg aaatagacag atcgctgaga 960

taggtgcctc actgattaag cattggtaac tgtcagacca agtttactca tatatacttt 1020

agattgattt aaaacttcat ttttaattta aaaggatcta ggtgaagatc ctttttgata 1080

atctcatgac caaaatccct taacgtgagt tttcgttcca ctgagcgtca gaccccgtag 1140

aaaagatcaa aggatcttct tgagatcctt ttttttctgcg cgtaatctgc tgcttgcaaa 1200

caaaaaaacc accgctacca gcggtggttt gtttgccgga tcaagagcta ccaactcttt 1260

ttccgaaggt aactggcttc agcagagcgc agataccaaa tactgtcctt ctagtgtagc 1320

cgtagttagg ccaccacttc aagaactctg tagcaccgcc tacatacctc gctctgctaa 1380

tcctgttacc agtggctgct gccagtggcg ataagtcgtg tcttaccggg ttggactcaa 1440

gacgatagtt accggataag gcgcagcggt cgggctgaac gggggggttcg tgcacacagc 1500

ccagcttgga gcgaacgacc tacaccgaac tgagatacct acagcgtgag ctatgagaaa 1560

gcgccacgct tcccgaaggg agaaaggcgg acaggtatcc ggtaagcggc agggtcggaa 1620

caggagagcg cacgagggag cttccagggg gaaacgcctg gtatctttat agtcctgtcg 1680

ggtttcgcca cctctgactt gagcgtcgat ttttgtgatg ctcgtcaggg gggcggagcc 1740

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agtgagctga taccgctcgc cgcagccgaa cgaccgagcg cagcgagtca gtgagcgagg 1920

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aagcgtaccc gataaaagcg gcttcctgac aggaggccgt tttgttttgc agcccaccta 1980

gcggaagagc gcccaatacg caaaccgcct ctccccgcgc gttggccgat tcattaatgc 2040

agctggcacg acaggtttcc cgactggaaa gcgggcagtg agcgcaacgc aattaatgtg 2100

agttagctca ctcattaggc accccaggct ttacacttta tgctcccggc tcgtatgttg 2160

tgtggaattg tgagcggata acaattgaat tcacatctag atatctcgag tcaatactag 2220

tggccaggcc ggccagcggc cgcattaccg atcgcccttc ccaacagttg cgcagcctga 2280

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cctttctcgc cacgttcgcc ggctttcccc gtcaagctct aaatcggggg ctccctttag 2460

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cacgtagtgg gccatcgccc tgatagacgg tttttcgccc tttgacgttg gagtccacgt 2580

tctttaatag tggactcttg ttccaaactg gaacaacact caaccctatc tcggtctatt 2640

cttttgattt ataagggatt ttgccgattt cggcctattg gttaaaaaat gagctgattt 2700

aacaaaaatt taacgcgaat tttaacaaaa tattaacgct tacaatttag 2750

<210> SEQ ID NO 22
<211> LENGTH: 102
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 22

aattcaagga gttaattatg aaaaaaaccg cgattgcgat tgcggtggcg ctggcgggct 60

ttgcgaccgt ggcccaggcg gcctctagaa tctgcggccg ca 102

<210> SEQ ID NO 23
<211> LENGTH: 102
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 23

ctagtgcggc cgcagattct agaggccgcc tgggccacgg tcgcaaagcc cgccagcgcc 60

accgcaatcg caatcgcggt ttttttcata attaactcct tg 102

<210> SEQ ID NO 24
<211> LENGTH: 565
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: stuffer sequence

<400> SEQUENCE: 24

tctagataac tgtggctgca ccatctgtct tcatcttccc gccatctgat gagcagttga 60

aatctggaac tgcctctgtt gtgtgcctgc tgaataactt ctatcccaga gaggccaaag 120

tacagtggaa ggtggataac gccctccaat cgggtaactc ccaggagagt gtcacagagc 180

aggacagcaa ggacagcacc tacagcctca gcagcaccct gacgctgagc aaagcagact 240

acgagaaaca caaagtctac gcctgcgaag tcacccatca gggcctgagc tccggaggtg 300

cctcagtcgt gtgcttcttg aacaacttct accccaaaga catcaatgtc aagtggaaga 360

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ttgatggcag tgaacgacaa aatggcgtcc tgaacagttg gactgatcag gacagcaaag 420

acagcaccta cagcatgagc agcaccctca cgttgaccaa ggacgagtat gaacgacata 480

acagctatac ctgtgaggcc actcacaaga catcaacttc acccattgtc aagagcttca 540

acaggaatga gtgttaagcg gccgc 565

<210> SEQ ID NO 25
<211> LENGTH: 1131
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: stuffer sequence

<400> SEQUENCE: 25

ctcgagctga tgagccatgg aagctgtgtc gcctgcacca ggctcccacg gctcgtggtg 60

cggtgcgctt ctggtgttcg ctgcctacag ccgacacgtc gagcttcgtg cccctagagt 120

tgcgcgtcac agcagcctcc ggcgctccgc gatatcaccg tgtcatccac atcaatgaag 180

tagtgctcct agacgccccc gtggggctgg tggcgcggtt ggctgacgag agcggccacg 240

tagtgttgcg ctggctcccg ccgcctgaga cacccatgac gtctcacatc cgctacgagg 300

tggacgtctc ggccggcaac ggcgcaggga gcgtacagag ggtggagatc ctggagggcc 360

gcaccgagtg tgtgctgagc aacctgcggg gccggacgcg ctacaccttc gccgtccgcg 420

cgcgtatggc tgagccgagc ttcggcggct tctggagcgc ctggtcggag cctgtgtcgc 480

tgctgacgcc tagcgacctg gacccccctca tcctgacgct ctccctcatc ctcgtggtca 540

tcctggtgct gctgaccgtg ctcgcgctgc tctcccaccg ccgggctctg aagcagaaga 600

tctggcctgg catcccgagc ccagagagcg agtttgaagg cctcttcacc acccacaagg 660

gtaacttcca gctgtggctg taccagaatg atggctgcct gtggtggagc ccctgcaccc 720

ccttcacgga ggacccacct gcttccctgg aagtcctctc agagcgctgc tgggggacga 780

tgcaggcagt ggagccgggg acagatgatg agggcccttt tcccctcgt ctcctgtgag 840

aattccccgt cggatacgag cagcgtggcc gttggctgcc tcgcacagga cttccttccc 900

gactccatca ctttctcctg gaaatacaag aacaactctg acatcagcag cacccggggc 960

ttcccatcag tcctgagagg gggcaagtac gcagccacct cacaggtgct gctgccttcc 1020

aaggacgtca tgcagggcac agacgaacac gtggtgtgca aagtccagca ccccaacggc 1080

aacaaagaaa agaacgtgcc tcttccagtg attgctgagc tgcctactag t 1131

<210> SEQ ID NO 26
<211> LENGTH: 1121
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: stuffer sequence

<400> SEQUENCE: 26

ctcgagctga tgagccatgg aagctgtgtc gcctgcacca ggctcccacg gctcgtggtg 60

cggtgcgctt ctggtgttcg ctgcctacag ccgacacgtc gagcttcgtg cccctagagt 120

tgcgcgtcac agcagcctcc ggcgctccgc gatatcaccg tgtcatccac atcaatgaag 180

tagtgctcct agacgccccc gtggggctgg tggcgcggtt ggctgacgag agcggccacg 240

tagtgttgcg ctggctcccg ccgcctgaga cacccatgac gtctcacatc cgctacgagg 300

tggacgtctc ggccggcaac ggcgcaggga gcgtacagag ggtggagatc ctggagggcc 360

gcaccgagtg tgtgctgagc aacctgcggg gccggacgcg ctacaccttc gccgtccgcg 420

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cgcgtatggc tgagccgagc ttcggcggct tctggagcgc ctggtcggag cctgtgtcgc 480

tgctgacgcc tagcgacctg gacccccтca tcctgacgct ctccctcatc ctcgtggtca 540

tcctggtgct gctgaccgtg ctcgcgctgc tctcccaccg ccgggctctg aagcagaaga 600

tctggcctgg catcccgagc ccagagagcg agtttgaagg cctcttcacc acccacaagg 660

gtaacttcca gctgtggctg taccagaatg atggctgcct gtggtggagc ccctgcaccc 720

ccttcacgga ggacccacct gcttccctgg aagtcctctc agagcgctgc tgggggacga 780

tgcaggcagt ggagccgggg acagatgatg agggcccatc ggtcttcccc ctggcaccct 840

cctccaagag cacctctggc ggcacagcgg ccctgggctg cctggtcaag gactacttcc 900

ccgaaccggt gacggtgtcg tggaactcag gcgctctgac cagcggcgtg cacaccttcc 960

cggctgtcct acagtcctca ggactctact ccctcagcag cgtggtgacc gtgccatcca 1020

gcagcttggg cacccagacc tacatctgca acgtgaatca caagcccagc aacaccaagg 1080

tggacaagaa agttgagccc aaatcttgtg acaaaactag t 1121

<210> SEQ ID NO 27
<211> LENGTH: 337
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: stuffer sequence

<400> SEQUENCE: 27

tctagataac tgtggctgca ccatctgtct tcatcttccc gccatctgat gagcagttga 60

aatctggaac tgcctctgtt gtgtgcctgc tgaataactt ctatcccaga gaggccaaag 120

tacagtggaa ggtggataac gccctccaat cgggtaactc ccaggagagt gtcacagagc 180

aggacagcaa ggacagcacc tacagcctca gcagcaccct gacgctgagc aaagcagact 240

acgagaaaca caaagtctac gcctgcgaag tcacccatca gggcctgagc tcgcccgtca 300

caaagagctt caacagggga gagtgttaag cggccgc 337

<210> SEQ ID NO 28
<211> LENGTH: 509
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: stuffer sequence

<400> SEQUENCE: 28

tctagataac tgtggctgca ccatctgtct tcatcttccc gccatctgat gagcagttga 60

aatctggaac tgcctctgtt gtgtgcctgc tgaataactt ctatcccaga gaggccaaag 120

tacagtggaa ggtggataac gccctccaat cgggtaactc ccaggagagt gtcacagagc 180

aggacagcaa ggacagcacc tacagcctca gcagcaccct gacgctgagc aaagcagact 240

acgagaaaca caaagtctac gcctgcgaag tcacccatca gggcctgagc tctgacagtg 300

gcttggaaag cagatagcag ccccgtcaag gcgggagtgg agaccaccac accctccaaa 360

caaagcaaca acaagtacgc ggccagcagc tatctgagcc tgacgcctga gcagtggaag 420

tcccacagaa gctacagctg ccaggtcacg catgaaggga gcaccgtgga gaagacagtg 480

gcccctacag aatgttcata agcggccgc 509

<210> SEQ ID NO 29
<211> LENGTH: 1059
<212> TYPE: DNA
<213> ORGANISM: artificial sequence

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<220> FEATURE:
<223> OTHER INFORMATION: stuffer sequence

<400> SEQUENCE: 29

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cggtgcgctt ctggtgttcg ctgcctacag ccgacacgtc gagcttcgtg cccctagagt 120

tgcgcgtcac agcagcctcc ggcgctccgc gatatcaccg tgtcatccac atcaatgaag 180

tagtgctcct agacgccccc gtggggctgg tggcgcggtt ggctgacgag agcggccacg 240

tagtgttgcg ctggctcccg ccgcctgaga cacccatgac gtctcacatc cgctacgagg 300

tggacgtctc ggccggcaac ggcgcaggga gcgtacagag ggtggagatc ctggagggcc 360

gcaccgagtg tgtgctgagc aacctgcggg gccggacgcg ctacaccttc gccgtccgcg 420

cgcgtatggc tgagccgagc ttcggcggct tctggagcgc ctggtcggag cctgtgtcgc 480

tgctgacgcc tagcgacctg gacccccctca tcctgacgct ctccctcatc ctcgtggtca 540

tcctggtgct gctgaccgtg ctcgcgctgc tctcccaccg ccgggctctg aagcagaaga 600

tctggcctgg catcccgagc ccagagagcg agtttgaagg cctcttcacc acccacaagg 660

gtaacttcca gctgtggctg taccagaatg atggctgcct gtggtggagc ccctgcaccc 720

ccttcacgga ggacccacct gcttccctgg aagtcctctc agagcgctgc tgggggacga 780

tgcaggcagt ggagccgggg acagatgatg agggccctag gatgcctggt caagggttat 840

ttccctgagc cagtgacctt gacctggaac tctggatccc tgtccagtgg tgtgcacacc 900

ttcccagctg tcctgcagtc tgacctctac accctcagca gctcagtgac tgtaacctcc 960

agcacctggc ccagccagtc catcacctgc aatgtggccc acccggcaag cagcaccaag 1020

gtggacaaga aaattgagcc cagagtgccc acaactagt 1059

<210> SEQ ID NO 30
<211> LENGTH: 1056
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: stuffer sequence

<400> SEQUENCE: 30

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cggtgcgctt ctggtgttcg ctgcctacag ccgacacgtc gagcttcgtg cccctagagt 120

tgcgcgtcac agcagcctcc ggcgctccgc gatatcaccg tgtcatccac atcaatgaag 180

tagtgctcct agacgccccc gtggggctgg tggcgcggtt ggctgacgag agcggccacg 240

tagtgttgcg ctggctcccg ccgcctgaga cacccatgac gtctcacatc cgctacgagg 300

tggacgtctc ggccggcaac ggcgcaggga gcgtacagag ggtggagatc ctggagggcc 360

gcaccgagtg tgtgctgagc aacctgcggg gccggacgcg ctacaccttc gccgtccgcg 420

cgcgtatggc tgagccgagc ttcggcggct tctggagcgc ctggtcggag cctgtgtcgc 480

tgctgacgcc tagcgacctg gacccccctca tcctgacgct ctccctcatc ctcgtggtca 540

tcctggtgct gctgaccgtg ctcgcgctgc tctcccaccg ccgggctctg aagcagaaga 600

tctggcctgg catcccgagc ccagagagcg agtttgaagg cctcttcacc acccacaagg 660

gtaacttcca gctgtggctg taccagaatg atggctgcct gtggtggagc ccctgcaccc 720

ccttcacgga ggacccacct gcttccctgg aagtcctctc agagcgctgc tgggggacga 780

tgcaggcagt ggagccgggg acagatgatg agggccctag gatgcctggt caagggctat 840

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ttccctgagc cagtgacagt gacctggaac tctggatccc tgtccagcgg tgtgcacacc 900

ttcccagctg tcctgcagtc tgacctctac actctgagca gctcagtgac tgtcccctcc 960

agcacctggc ccagcgagac cgtcacctgc aacgttgccc acccggccag cagcaccaag 1020

gtggacaaga aaattgtgcc cagggattgt actagt 1056

<210> SEQ ID NO 31
<211> LENGTH: 4153
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: plasmid vector

<400> SEQUENCE: 31

gtggcacttt tcggggaaat gtgcgcggaa cccctatttg tttatttttc taaatacatt 60

caaatatgta tccgctcatg agacaataac cctgataaat gcttcaataa tattgaaaaa 120

ggaagagtat gagtattcaa catttccgtg tcgcccttat tccctttttt gcggcatttt 180

gccttcctgt tttgctcac ccagaaacgc tggtgaaagt aaaagatgct gaagatcagt 240

tgggtgcacg agtgggttac atcgaactgg atctcaacag cggtaagatc cttgagagtt 300

ttcgccccga agaacgtttt ccaatgatga gcacttttaa agttctgcta tgtggcgcgg 360

tattatcccg tattgacgcc gggcaagagc aactcggtcg ccgcatacac tattctcaga 420

atgacttggt tgagtactca ccagtcacag aaaagcatct tacggatggc atgacagtaa 480

gagaattatg cagtgctgcc ataaccatga gtgataacac tgcggccaac ttacttctga 540

caacgatcgg aggaccgaag gagctaaccg ctttttttgca caacatgggg gatcatgtaa 600

ctcgccttga tcgttgggaa ccggagctga atgaagccat accaaacgac gagcgtgaca 660

ccacgatgcc tgtagcaatg gcaacaacgt tgcgcaaact attaactggc gaactactta 720

ctctagcttc ccggcaacaa ttaatagact ggatggaggc ggataaagtt gcaggaccac 780

ttctgcgctc ggcccttccg gctggctggt ttattgctga taaatctgga gccggtgagc 840

gtgggtctcg cggtatcatt gcagcactgg ggccagatgg taagccctcc cgtatcgtag 900

ttatctacac gacggggagt caggcaacta tggatgaacg aaatagacag atcgctgaga 960

taggtgcctc actgattaag cattggtaac tgtcagacca agtttactca tatatacttt 1020

agattgattt aaaacttcat ttttaattta aaaggatcta ggtgaagatc ctttttgata 1080

atctcatgac caaaatccct taacgtgagt tttcgttcca ctgagcgtca gaccccgtag 1140

aaaagatcaa aggatcttct tgagatcctt ttttttctgcg cgtaatctgc tgcttgcaaa 1200

caaaaaaacc accgctacca gcggtggttt gtttgccgga tcaagagcta ccaactcttt 1260

ttccgaaggt aactggcttc agcagagcgc agataccaaa tactgtcctt ctagtgtagc 1320

cgtagttagg ccaccacttc aagaactctg tagcaccgcc tacatacctc gctctgctaa 1380

tcctgttacc agtggctgct gccagtggcg ataagtcgtg tcttaccggg ttggactcaa 1440

gacgatagtt accggataag gcgcagcggt cgggctgaac gggggttcg tgcacacagc 1500

ccagcttgga gcgaacgacc tacaccgaac tgagatacct acagcgtgag ctatgagaaa 1560

gcgccacgct tcccgaaggg agaaaggcgg acaggtatcc ggtaagcggc agggtcggaa 1620

caggagagcg cacgagggag cttccagggg gaaacgcctg gtatctttat agtcctgtcg 1680

ggtttcgcca cctctgactt gagcgtcgat ttttgtgatg ctcgtcaggg gggcggagcc 1740

tatggaaaaa cgccagcaac gcggcctttt tacggttcct ggccttttgc tggccttttg 1800

ctcacatgtt ctttcctgcg ttatcccctg attctgtgga taaccgtatt accgcctttg 1860

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aagcgtaccc gataaaagcg gcttcctgac aggaggccgt tttgttttgc agcccaccta 1980

gcggaagagc gcccaatacg caaaccgcct ctccccgcgc gttggccgat tcattaatgc 2040

agctggcacg acaggtttcc cgactggaaa gcgggcagtg agcgcaacgc aattaatgtg 2100

agttagctca ctcattaggc accccaggct ttacacttta tgctcccggc tcgtatgttg 2160

tgtggaattg tgagcggata acaattgaat tcaaggagtt aattatgaaa aaaaccgcga 2220

ttgcgattgc ggtggcgctg gcgggctttg cgaccgtggc ccaggcggcc tctagaatct 2280

gcggccgcac tagatataat taaggagata aatatgaaat atctgctgcc gaccgcggcg 2340

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aggccggcct tgaaactgtt gaaagttgtt tagcaaaacc ccatacagaa aattcattta 2460

ctaacgtctg gaaagacgac aaaactttag atcgttacgc taactatgag ggctgtctgt 2520

ggaatgctac aggcgttgta gtttgtactg gtgacgaaac tcagtgttac ggtacatggg 2580

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cgggctatac ttatatcaac cctctcgacg gcacttatcc gcctggtact gagcaaaacc 2760

ccgctaatcc taatccttct cttgaggagt ctcagcctct taatactttc atgtttcaga 2820

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attttgctgg ctctaattcc caaatggctc aagtcggtga cggtgataat tcacctttaa 3420

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tctttggcgc tggtaaacca tatgaatttt ctattgattg tgacaaaata aacttattcc 3540

gtggtgtctt tgcgtttctt ttatatgttg ccacctttat gtatgtattt tcgacgtttg 3600

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ttcccaacag ttgcgcagcc tgaatggcga atgggacgcg ccctgtagcg gcgcattaag 3720

cgcggcgggt gtggtggtta cgcgcagcgt gaccgctaca cttgccagcg ccctagcgcc 3780

cgctcctttc gctttcttcc cttcctttct cgccacgttc gccggctttc cccgtcaagc 3840

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aaaacttgat tagggtgatg gttcacgtag tgggccatcg ccctgataga cggtttttcg 3960

ccctttgacg ttggagtcca cgttctttaa tagtggactc ttgttccaaa ctggaacaac 4020

actcaaccct atctcggtct attcttttga tttataaggg attttgccga tttcggccta 4080

ttggttaaaa aatgagctga tttaacaaaa atttaacgcg aattttaaca aaatattaac 4140

gcttacaatt tag 4153

We claim:

[1. A phagemid vector comprising:
a selectable marker;
a ColE1 origin;
an f1 origin; and
after the ColE1 origin but before the f1 origin, further comprising the following features:
a bacterial transcription terminator;
a promoter,
a first ribosomal binding site;
a first leader sequence;
a first cloning region;
a second ribosomal binding site;
a second leader sequence;
a second cloning region for receiving a gene encoding a polypeptide to be displayed; and
a nucleotide sequence encoding a product that enables display of a polypeptide on the surface of a phagemid particle.]

[2. A phagemid vector as in claim 1 wherein at least one of the first or second ribosomal binding sites comprises Seq. ID No. 13.]

3. [A phagemid vector as in claim 1] *A phagemid vector comprising:*

a selectable marker;

a ColE1 origin;

an f1 origin; and

after the ColE1 origin but before the f1 origin, further comprising the following features: a bacterial transcription terminator and a promoter wherein the bacterial transcription terminator is upstream of the promoter;

a first ribosomal binding site;

a first leader sequence;

a first cloning region;

a second ribosomal binding site;

a second leader sequence;

a second cloning region for receiving a gene encoding a polypeptide to be displayed; and

a nucleotide sequence encoding a product that enables display of a polypeptide on the surface of a phagemid particle,

wherein at least one of the first or second leader sequences comprises a sequence selected from the group consisting of Seq. ID No. 14 and Seq. ID No. 17.

[4. A phagemid vector as in claim 1 wherein the nucleotide sequence encoding a product encodes a protein selected from the group consisting of pIII and pVIII.]

[5. A phagemid vector as in claim 1 wherein the nucleotide sequence encoding a product encodes a truncated pIII.]

[6. A phagemid vector as in claim 1 wherein the nucleotide sequence encoding a product encodes a synthetic pIII.]

[7. A phagemid vector as in claim 1 wherein the selectable marker is selected from the group consisting of ampicillin resistance, chloramphenicol transferase resistance, tetracycline resistance and kanamycin resistance.]

8. A phagemid vector comprising Seq. ID No. 18.

9. A vector comprising a sequence selected from the group consisting of Seq. ID Nos. 19, 20 and 21.

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