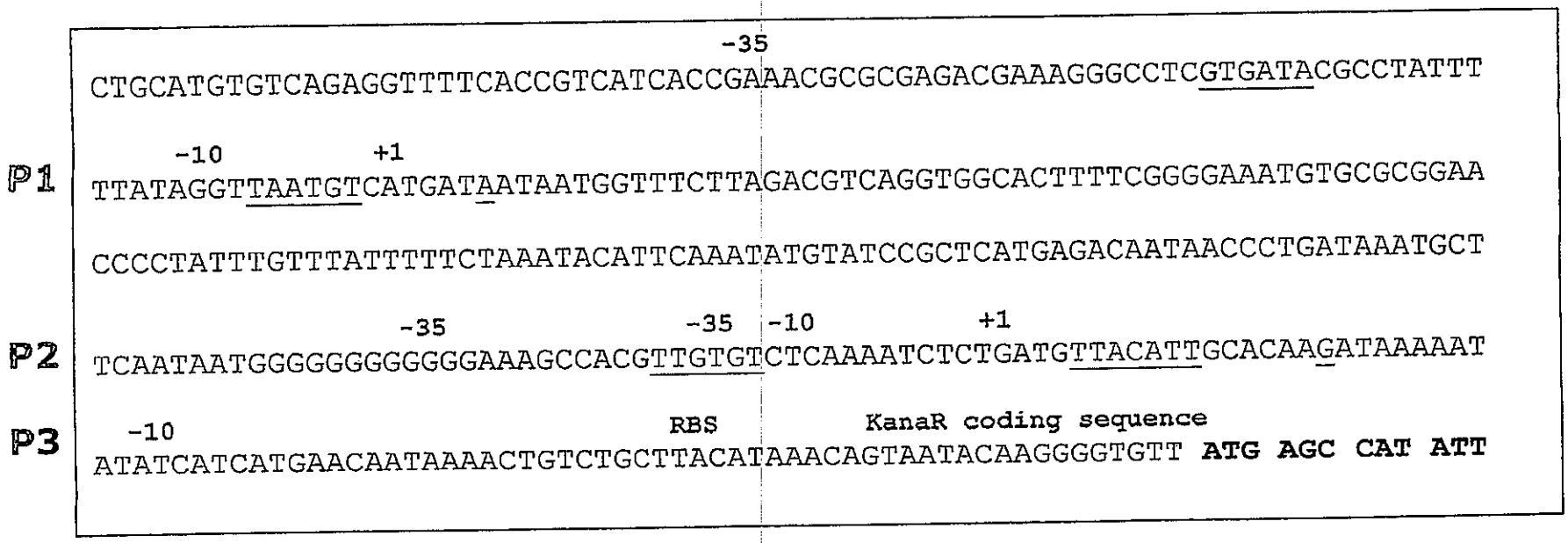

-35
CTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTT
-10
+1
P1
TTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAA
CCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCT
-35
-35
-10
+1
P2
TCAATAATGGGGGGGGGGGGAAAGCCACGTTGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAAT
-10
RBS
KanaR coding sequence
P3
ATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTT ATG AGC CAT ATT

FIG. 1

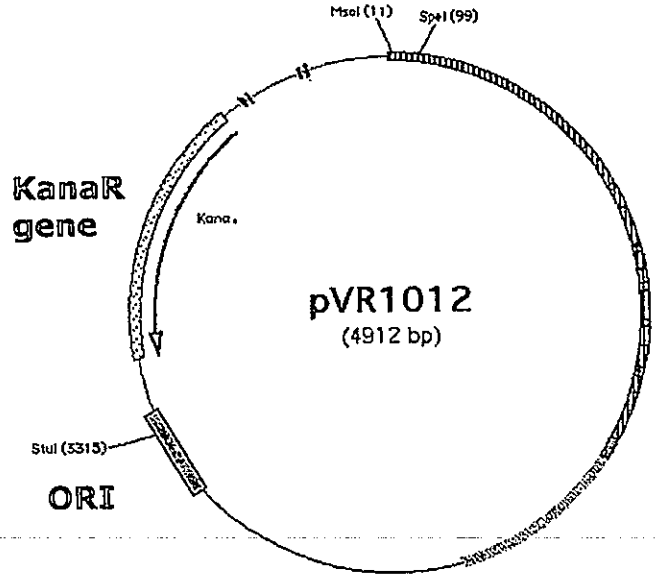


FIG. 2

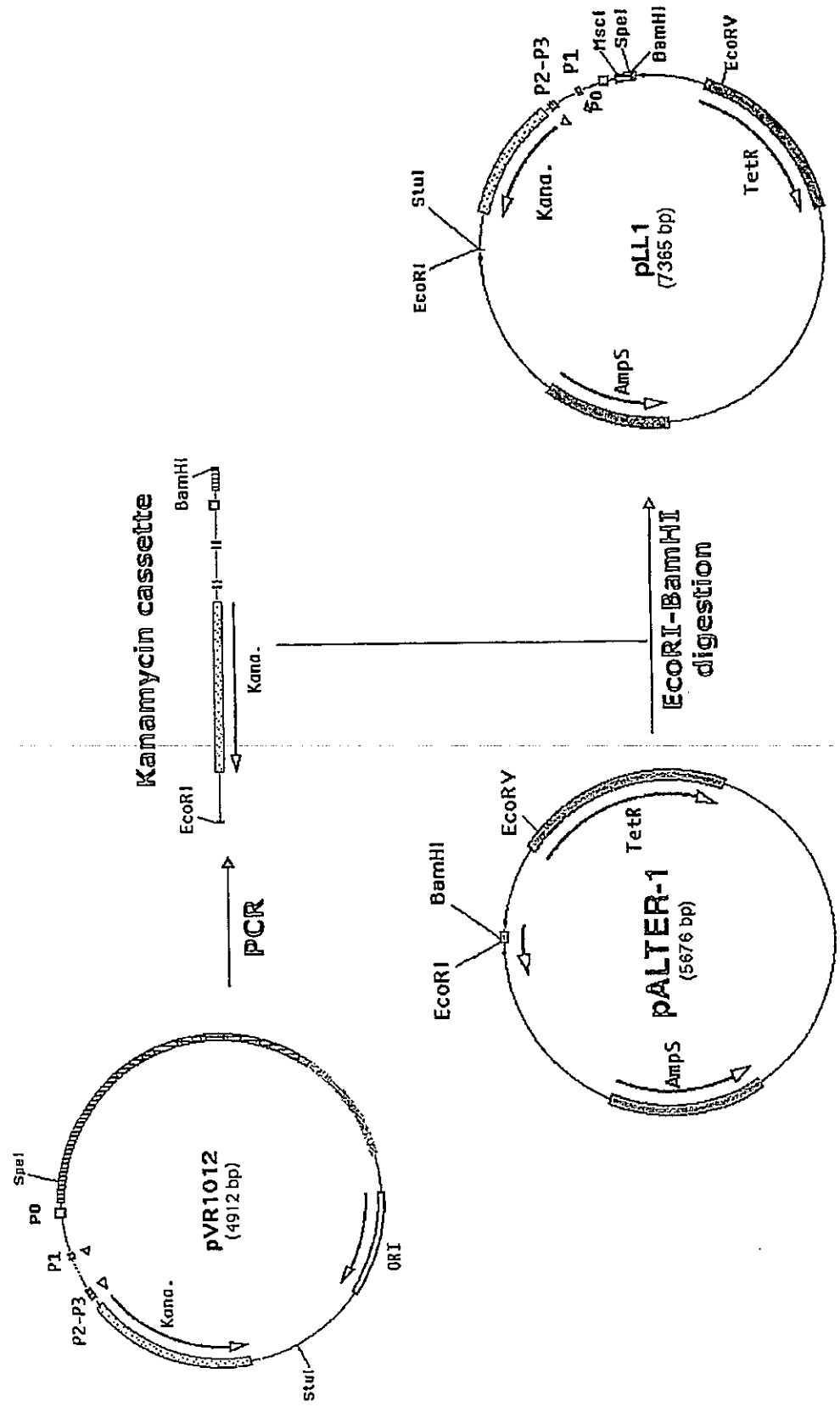

pVR1012
(4912 bp)
SpeI
P0
P1
P2-P3
Kana.
StuI
ORI
PCR
Kanamycin cassette
BamHI
EcoRI
Kana.
EcoRI-BamHI
digestion
pALTER-1
(5676 bp)
EcoRI
BamHI
EcoRV
TetR
AmpS
pLL1
(7365 bp)
EcoRI
StuI
P2-P3
P1
P0
MscI
SpeI
BamHI
EcoRV
Kana.
TetR
AmpS

FIG. 3

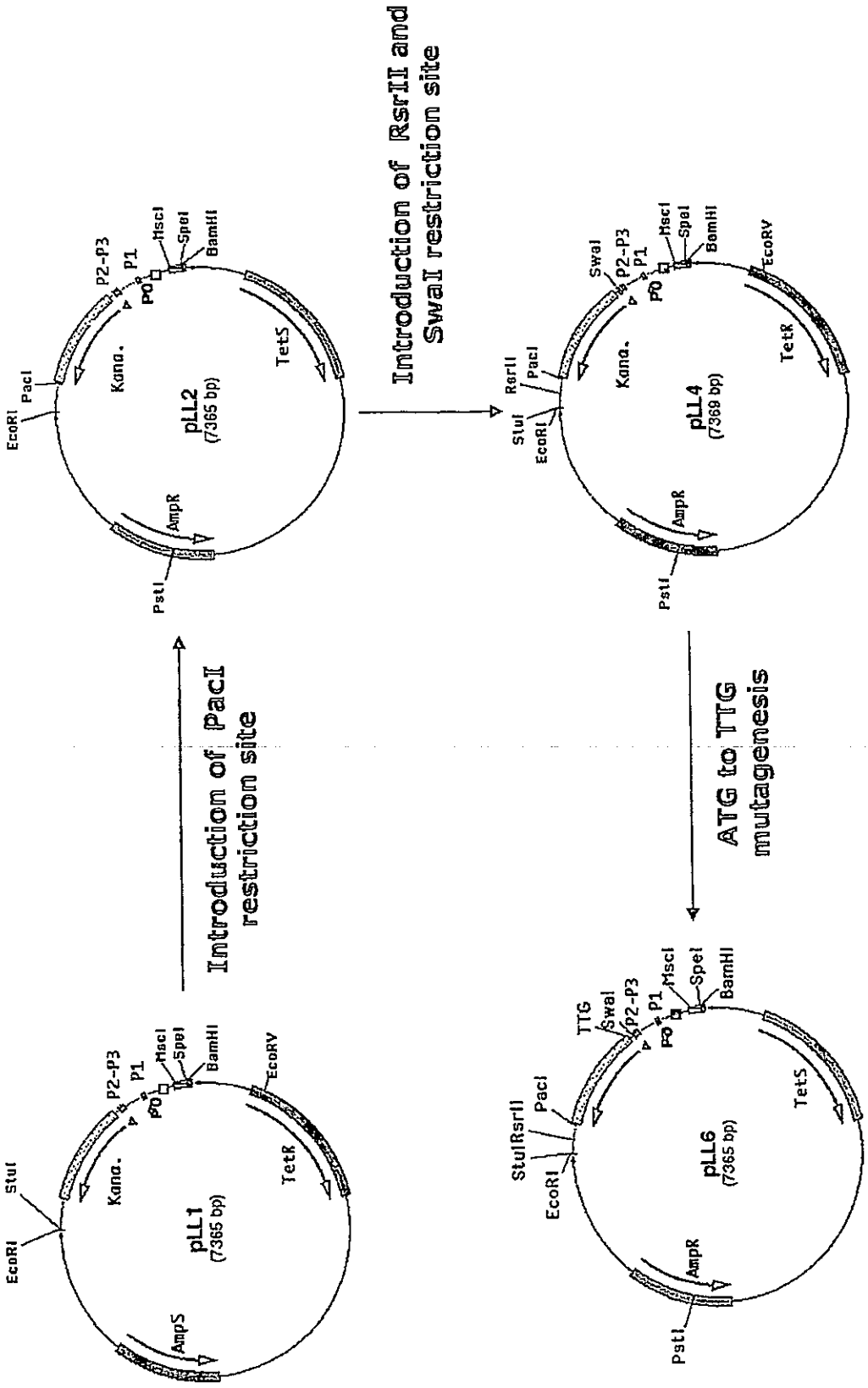

pLL1
(7365 bp)
pLL2
(7365 bp)
pLL4
(7369 bp)
pLL6
(7365 bp)
Introduction of PacI restriction site
Introduction of RsrII and SwaI restriction site
ATG to TTG mutagenesis
EcoRI
StuI
PacI
RsrII
SwaI
TTG
P2-P3
P1
PO
MscI
SpeI
BamHI
EcoRV
TetR
TetS
AmpS
AmpR
PstI
Kana.

FIG. 4

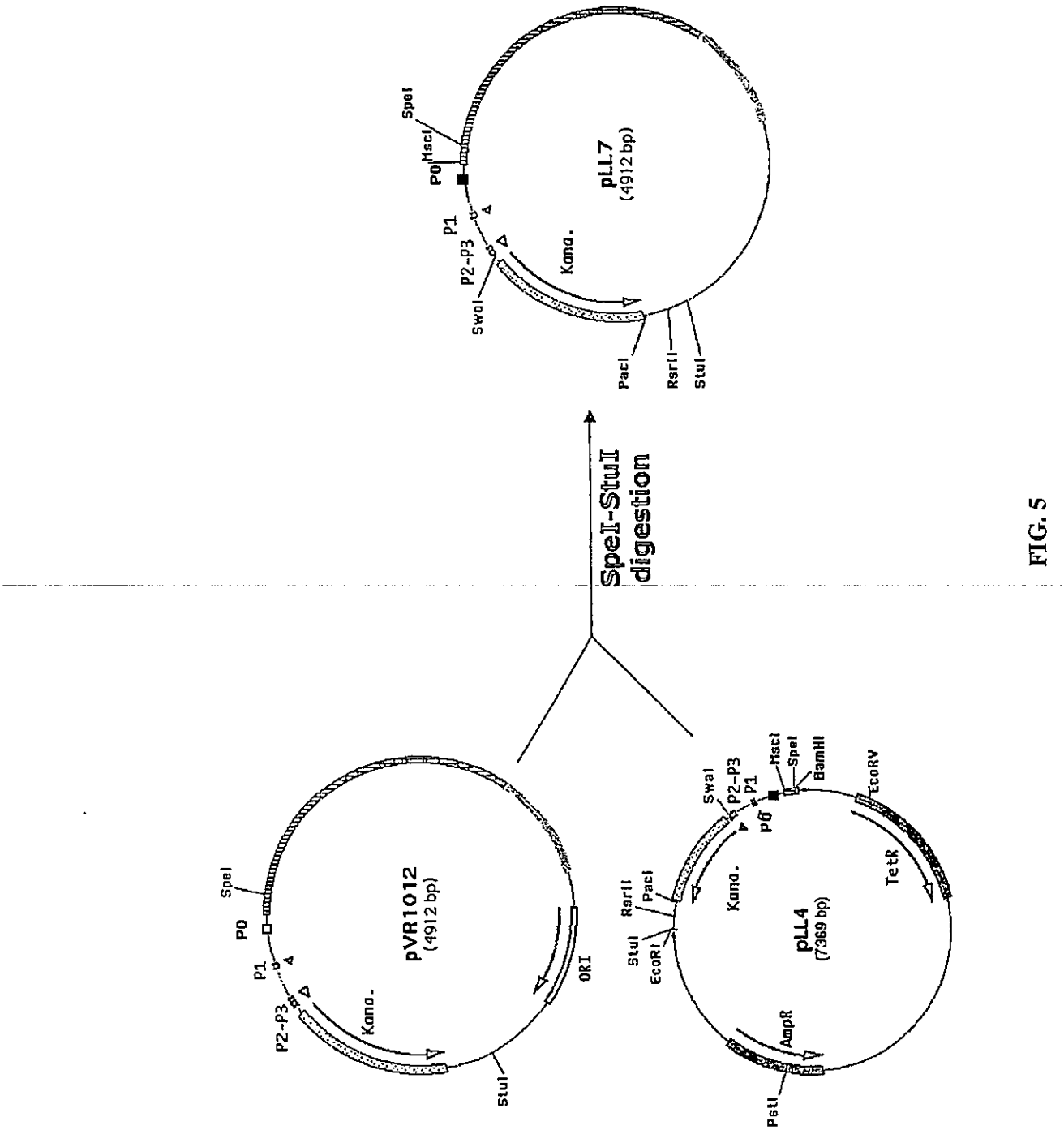

SpeI-StuI digestion
pVR1012
(4912 bp)
SpeI
P0
P1
P2-P3
Kana.
StuI
ORI
pLL7
(4912 bp)
SpeI
MscI
P0
P1
P2-P3
SwaI
Kana.
PacI
RsrII
StuI
pLL4
(7369 bp)
SwaI
P2-P3
P1
P0
MscI
SpeI
BamHI
EcoRV
TetR
AapR
PstI
StuI
EcoRI
RsrII
PacI
Kana.

FIG. 5

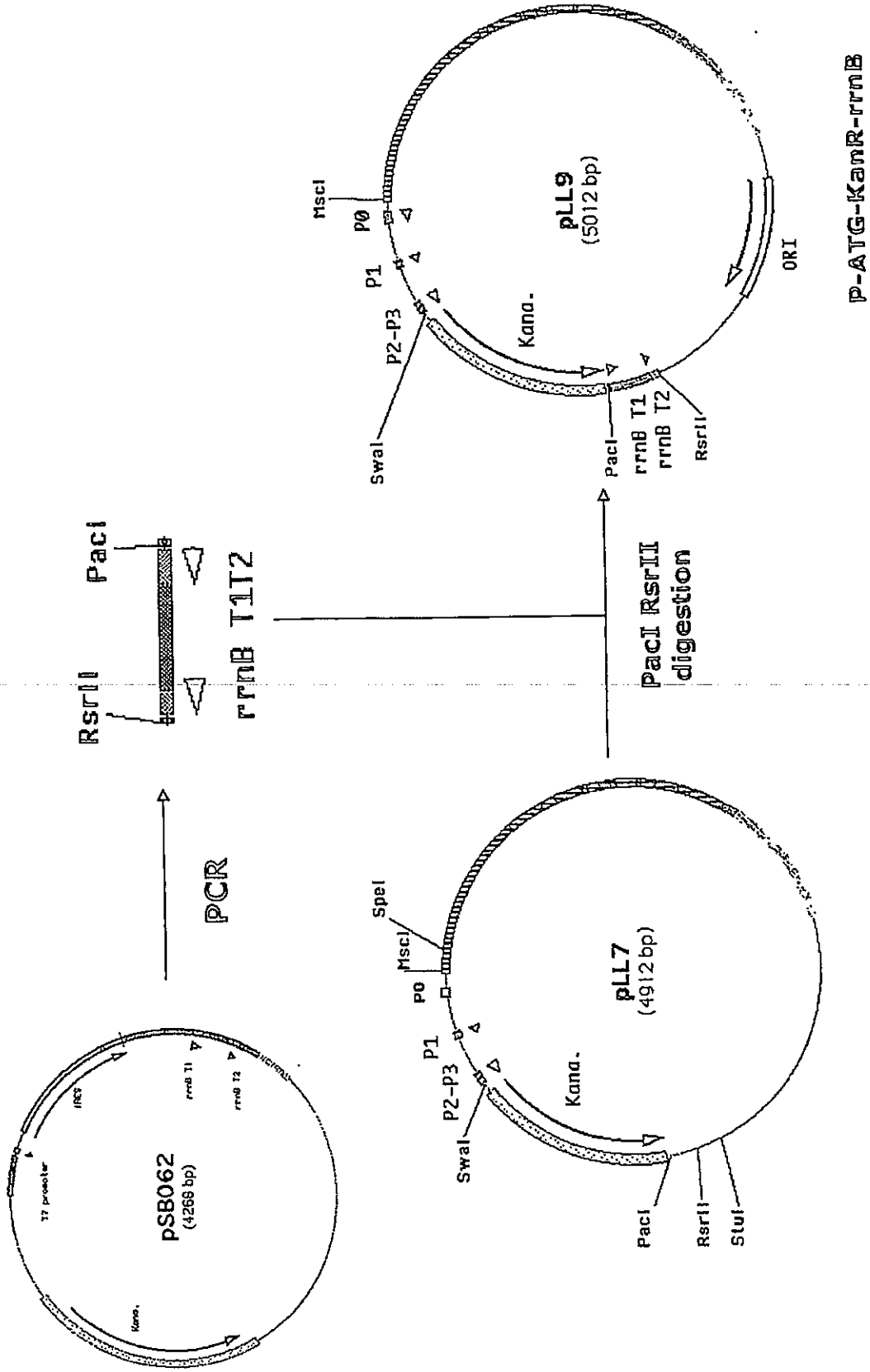

pSB062
(4268 bp)
T7 promoter
RBS
rrnB T1
rrnB T2
Kana.
PCR
RsrII
PacI
rrnB T1T2
pLL7
(4912 bp)
SpeI
MscI
P0
P1
P2-P3
SwaI
Kana.
PacI
RsrII
StuI
PacI RsrII
digestion
pLL9
(5012 bp)
MscI
P0
P1
P2-P3
SwaI
Kana.
PacI
rrnB T1
rrnB T2
RsrII
ORI
P-ATG-KanR-rrnB

FIG. 6

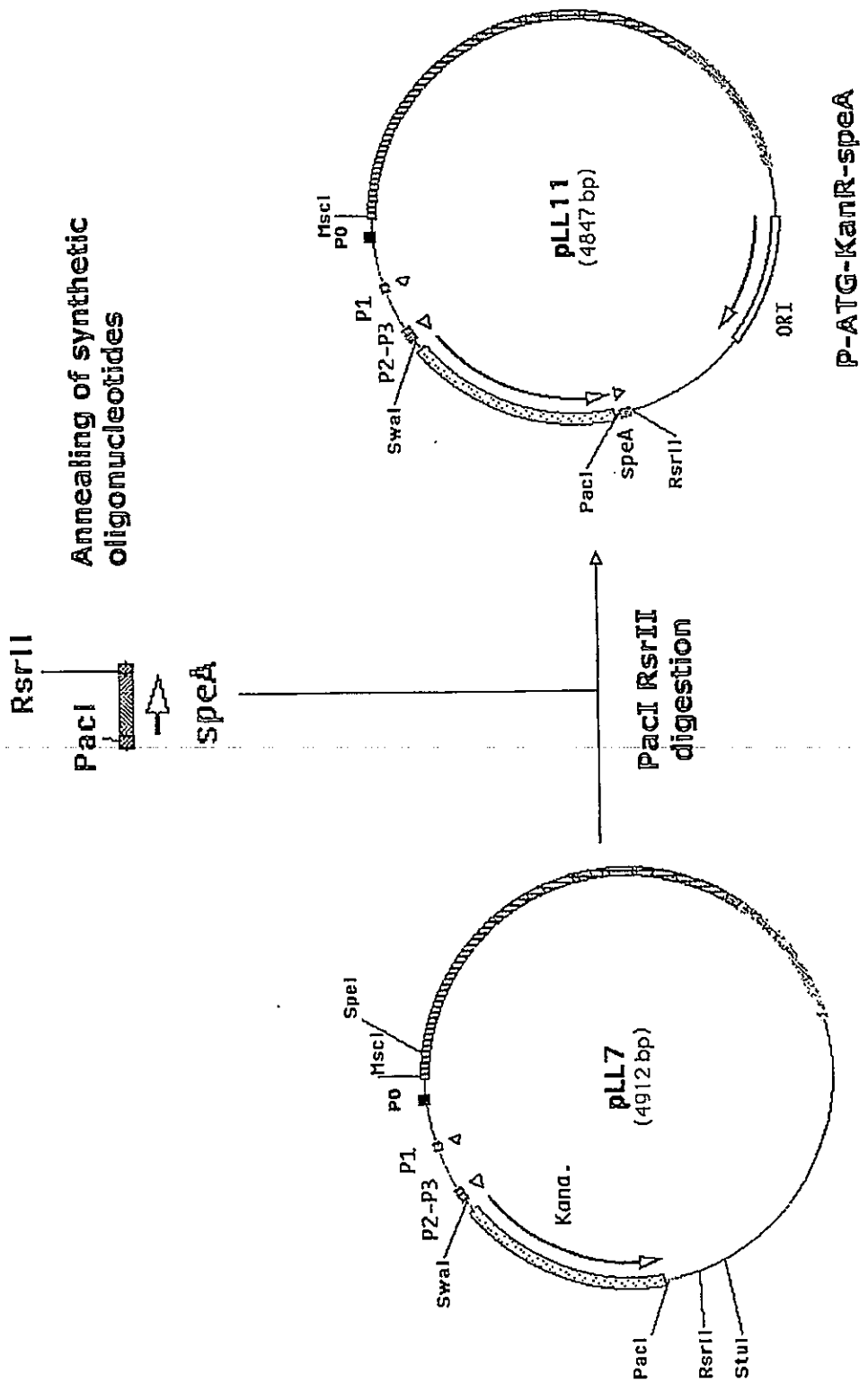

RsrII
PacI
speA
Annealing of synthetic oligonucleotides
PacI RsrII digestion
pLL7
(4912 bp)
SpeI
MscI
P0
P1
P2-P3
SwaI
Kana.
PacI
RsrII
StuI
pLL11
(4847 bp)
MscI
P0
P1
P2-P3
SwaI
speA
PacI
RsrII
ORI
P-ATG-KanR-speA

FIG. 7

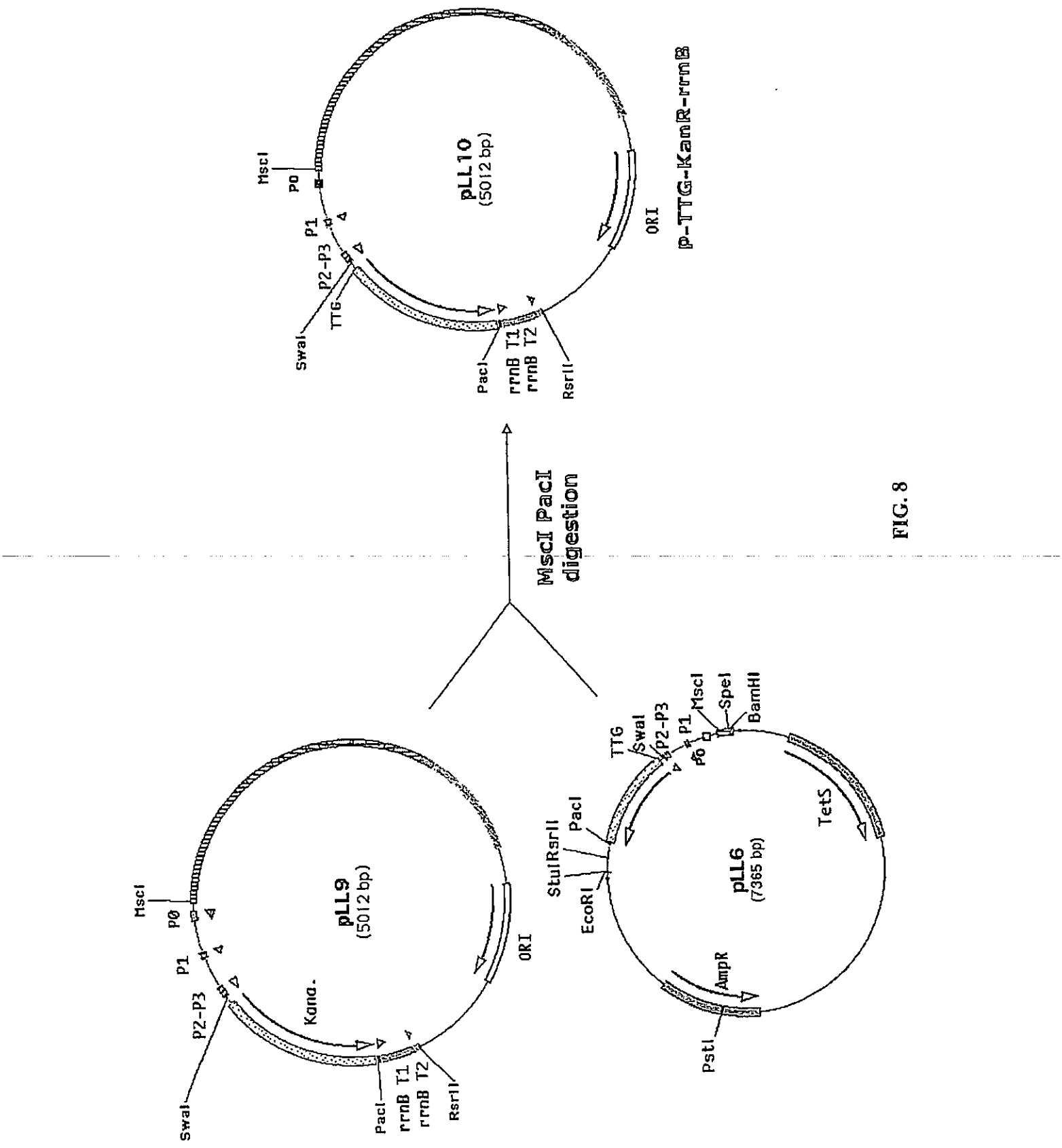

pLL10
(5012 bp)
MscI
P0
P1
P2-P3
TTG
SwaI
PacI
rrnB T1
rrnB T2
RsrII
ORI
P-TTG-KamR-rrnB
MscI PacI
digestion
pLL9
(5012 bp)
MscI
P0
P1
P2-P3
SwaI
Kana.
PacI
rrnB T1
rrnB T2
RsrII
ORI
pLL6
(7365 bp)
TTG
SwaI
P2-P3
P1
P0
MscI
SpeI
BamHI
TetS
StuI RsrII
PacI
EcoRI
AmpR
PstI

FIG. 8

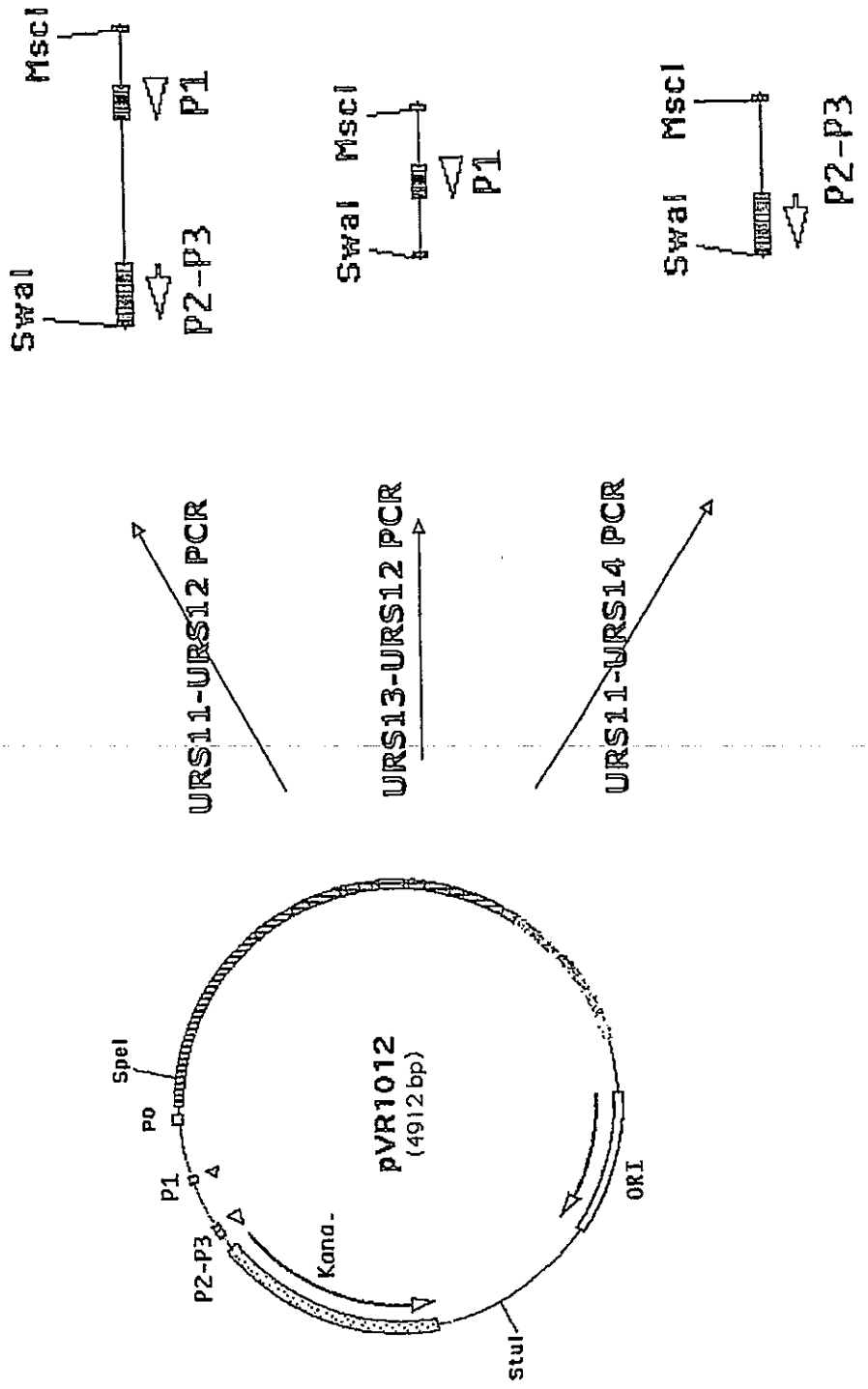

SwaI
MscI
P1
P2-P3
SwaI
MscI
P1
SwaI
MscI
P2-P3
URS11-URS12 PCR
URS13-URS12 PCR
URS11-URS14 PCR
pVR1012
(4912 bp)
SpeI
Po
P1
P2-P3
Kana-
ORI
StuI

FIG. 9

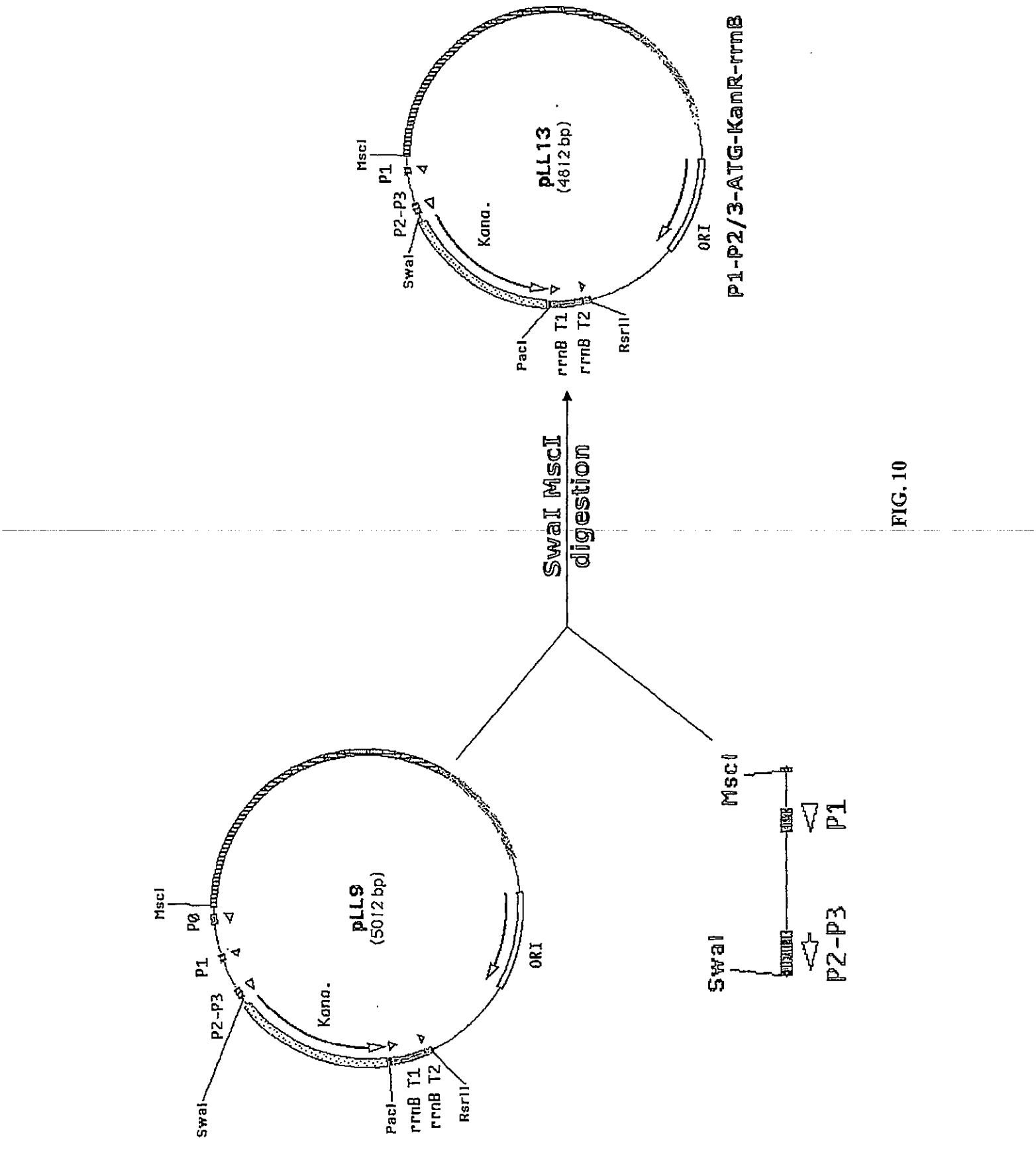

MscI
P0
P1
P2-P3
SwaI
Kana.
pLL9
(5012 bp)
ORI
PacI
rrnB T1
rrnB T2
RsrII
SwaI MscI
digestion
MscI
P1
P2-P3
SwaI
MscI
P1
P2-P3
SwaI
Kana.
pLL13
(4812 bp)
ORI
PacI
rrnB T1
rrnB T2
RsrII
P1-P2/3-ATG-KanR-rrnB

FIG. 10

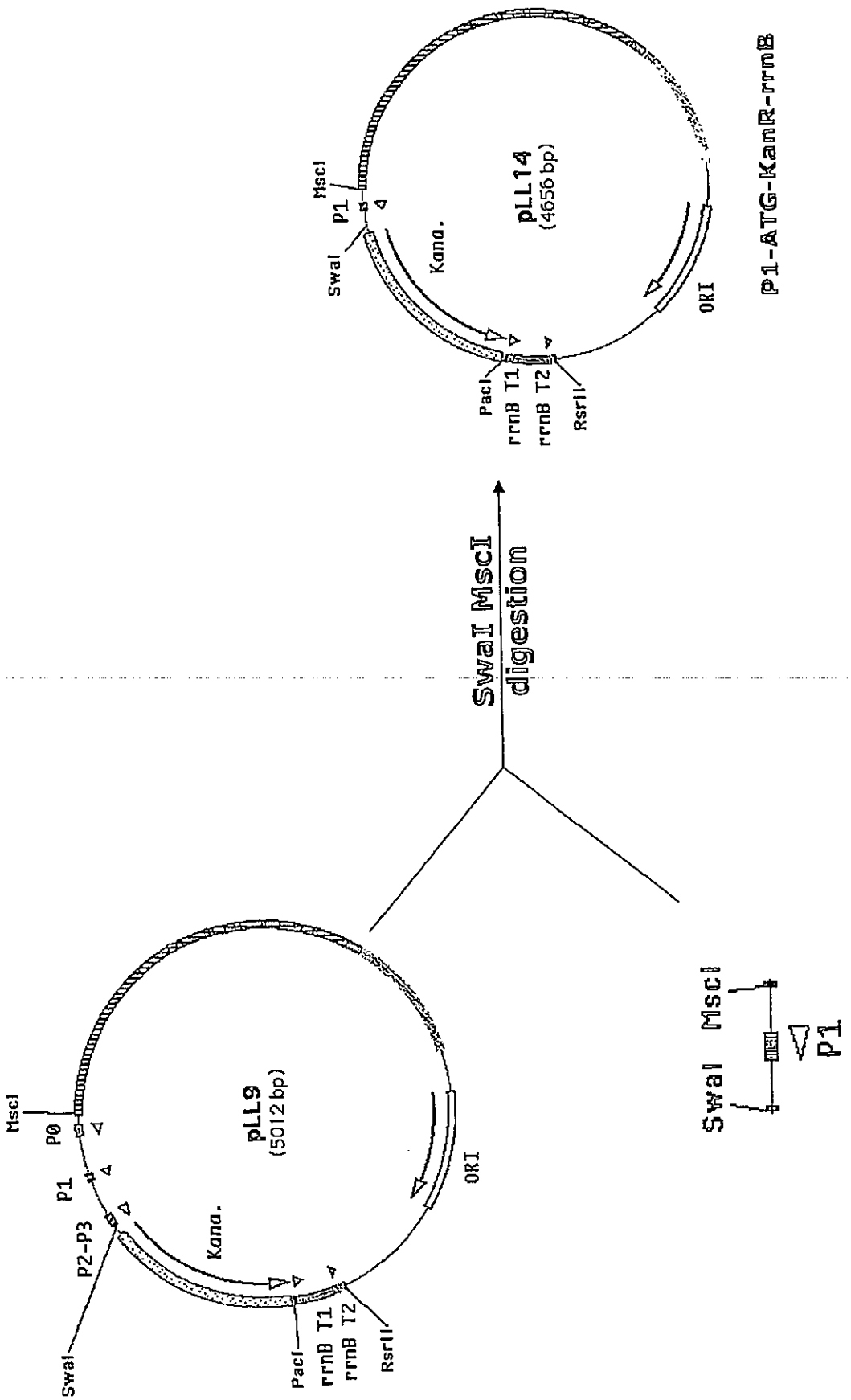

MscI
P0
P1
P2-P3
SwaI
pLL9
(5012 bp)
Kana.
ORI
PacI
rrnB T1
rrnB T2
RsrII
SwaI MscI
digestion
MscI
P1
SwaI
pLL14
(4656 bp)
Kana.
ORI
PacI
rrnB T1
rrnB T2
RsrII
P1-ATG-KanR-rrnB
SwaI MscI
P1

FIG. 11

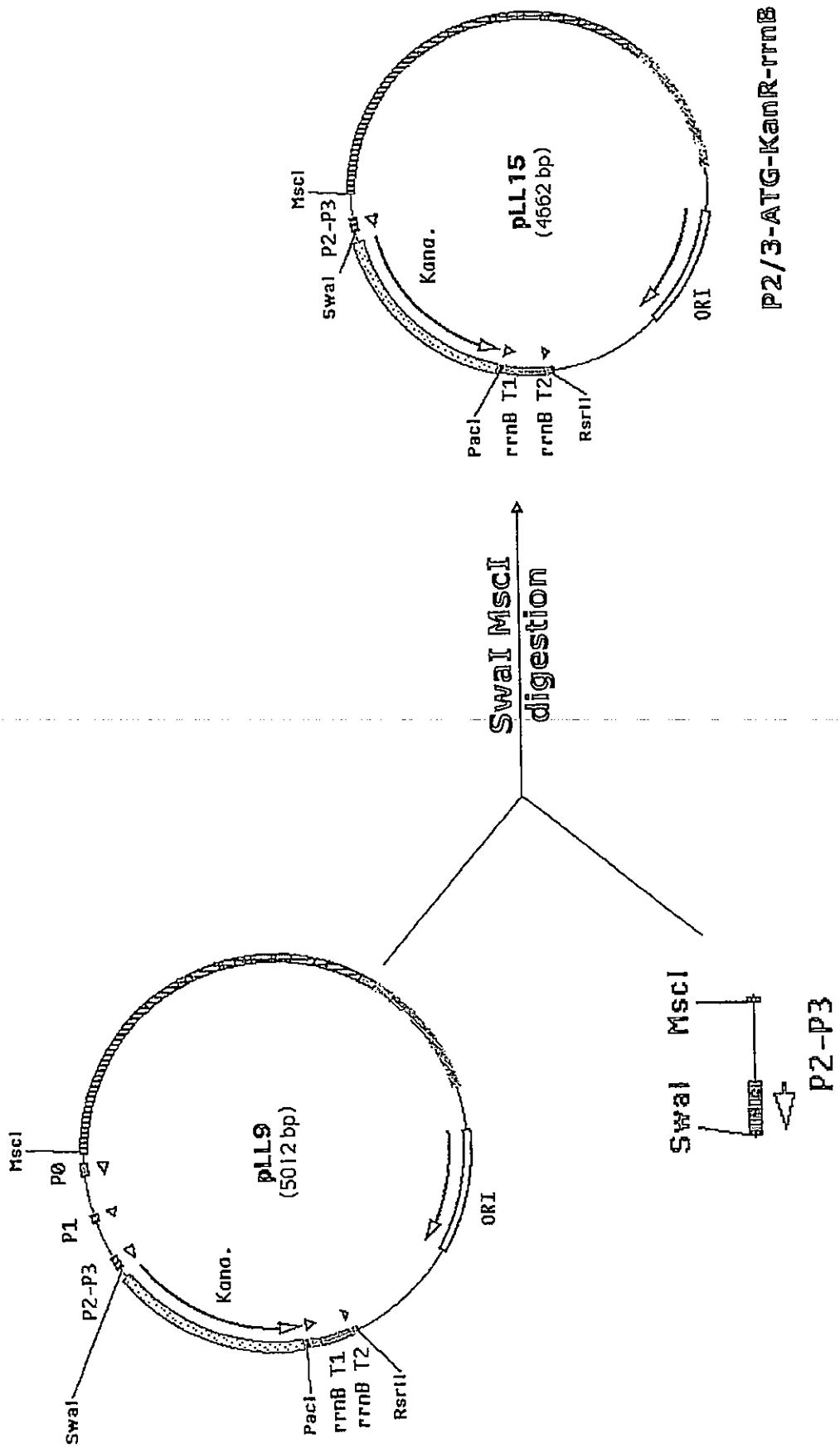

MscI
P0
P1
P2-P3
Kana.
pLL9
(5012 bp)
ORI
SwaI
PacI
rrnB T1
rrnB T2
RsrII
SwaI MscI
digestion
SwaI
MscI
P2-P3
MscI
P2-P3
SwaI
Kana.
pLL15
(4662 bp)
ORI
PacI
rrnB T1
rrnB T2
RsrII
P2/3-ATG-KanR-rrnB

FIG. 12

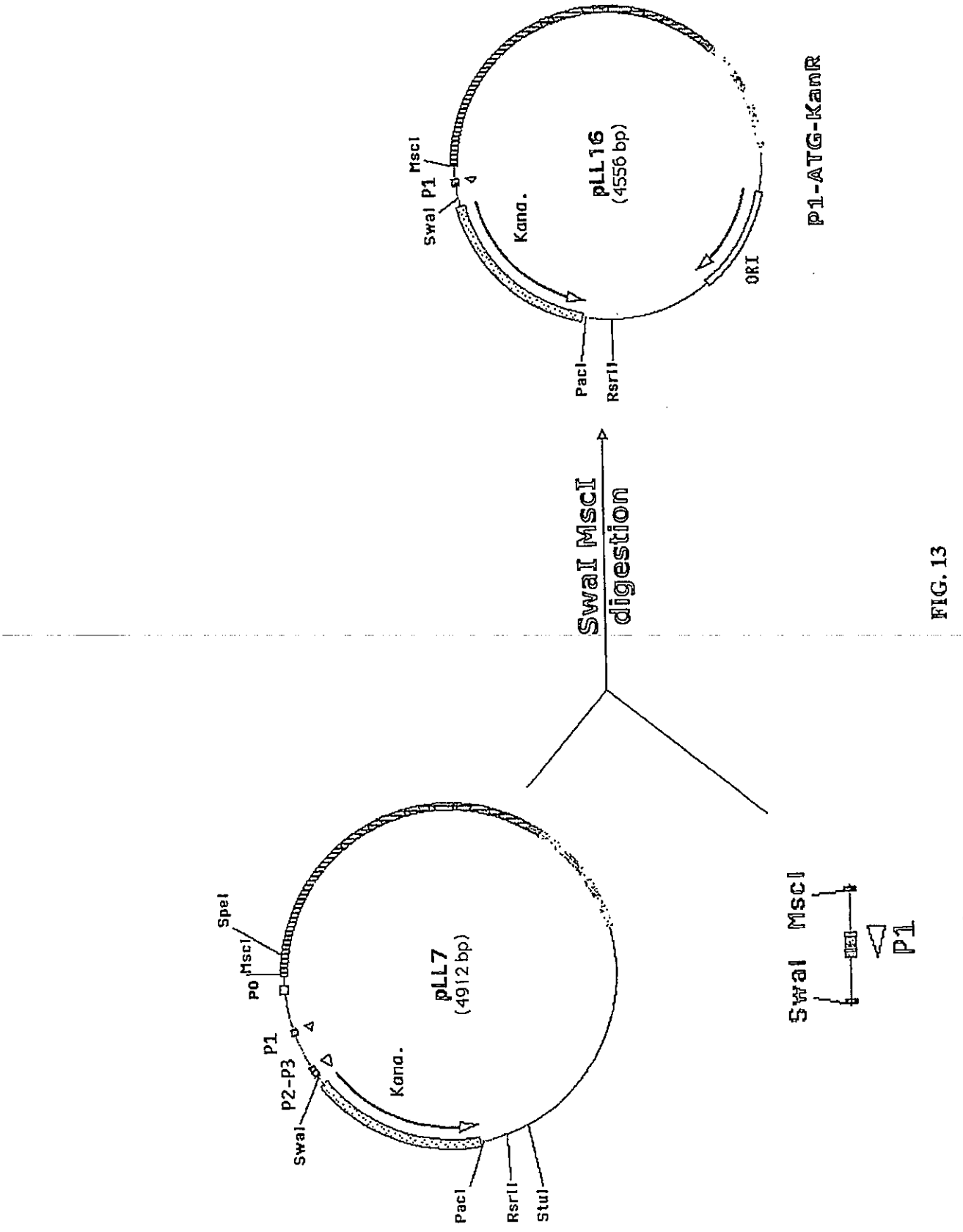
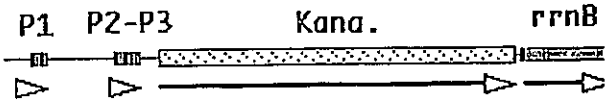

pLL16
(4556 bp)
MscI
SwaI P1
Kana.
ORI
PacI
RsrII
P1-ATG-KanR
SwaI MscI
digestion
pLL7
(4912 bp)
SpeI
P0
MscI
P1
P2-P3
SwaI
Kana.
PacI
RsrII
StuI
SwaI MscI
P1

FIG. 13



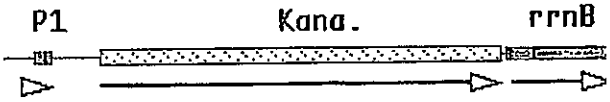
pVR1012



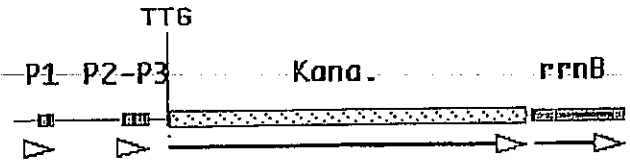
pLL13



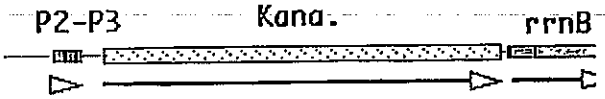
pLL9



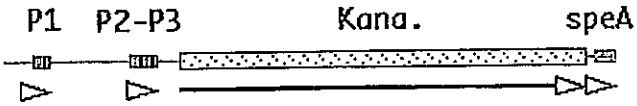
pLL14



pLL10



pLL15



pLL11



pLL16

FIG. 14

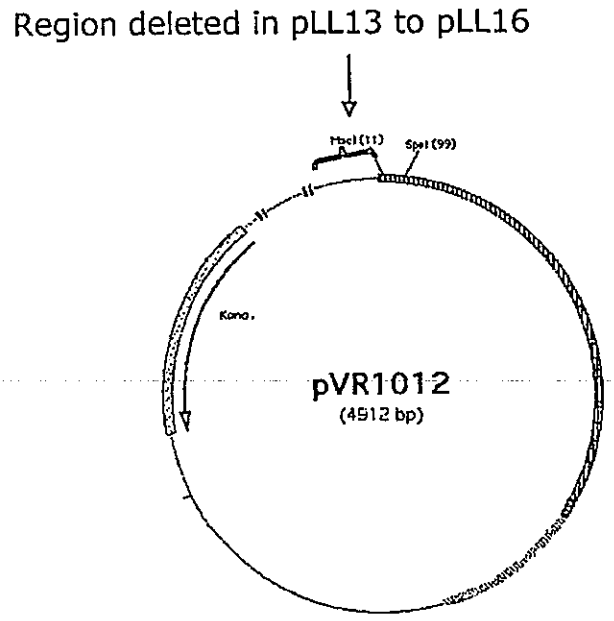
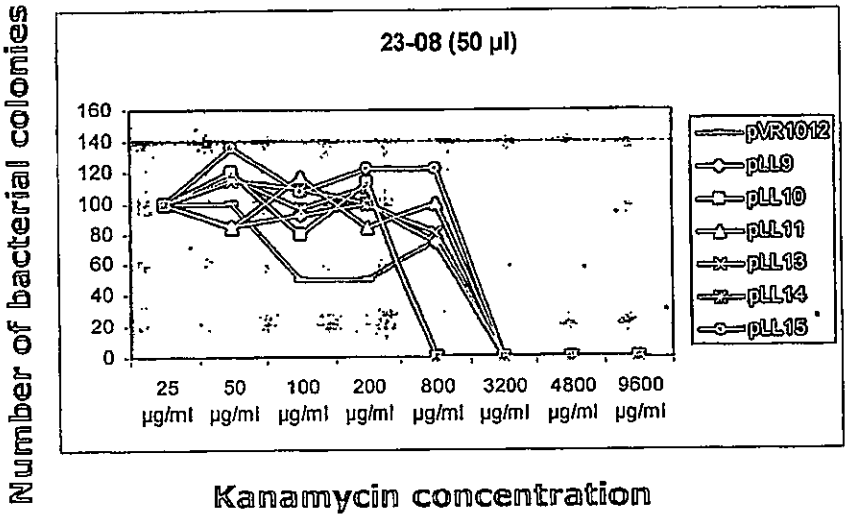

Region deleted in pLL13 to pLL16
HindIII(11)
SpeI(99)
Kana.
pVR1012
(4912 bp)

FIG. 15


23-08 (50 µl)
Number of bacterial colonies
160
140
120
100
80
60
40
20
0
25 µg/ml
50 µg/ml
100 µg/ml
200 µg/ml
800 µg/ml
3200 µg/ml
4800 µg/ml
9600 µg/ml
pVR1012
pLL9
pLL10
pLL11
pLL13
pLL14
pLL15
Kanamycin concentration

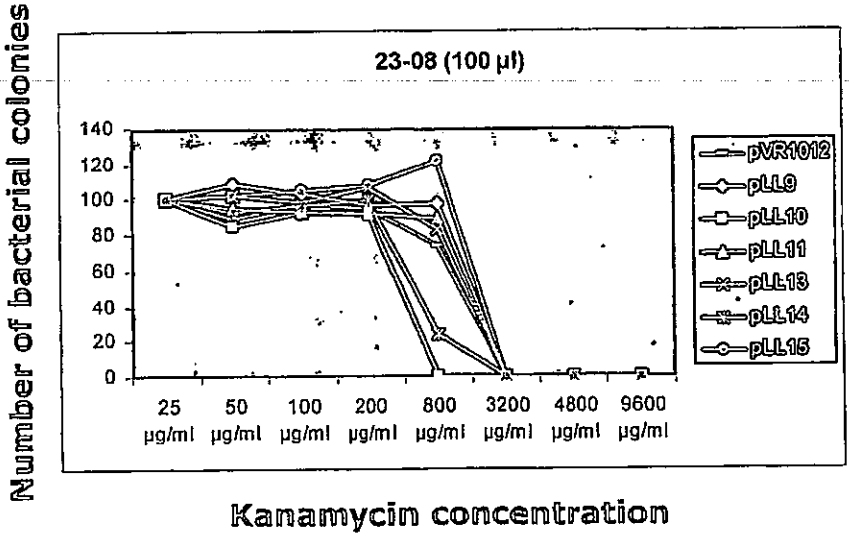

23-08 (100 µl)
Number of bacterial colonies
140
120
100
80
60
40
20
0
25 µg/ml
50 µg/ml
100 µg/ml
200 µg/ml
800 µg/ml
3200 µg/ml
4800 µg/ml
9600 µg/ml
pVR1012
pLL9
pLL10
pLL11
pLL13
pLL14
pLL15
Kanamycin concentration

FIG. 16

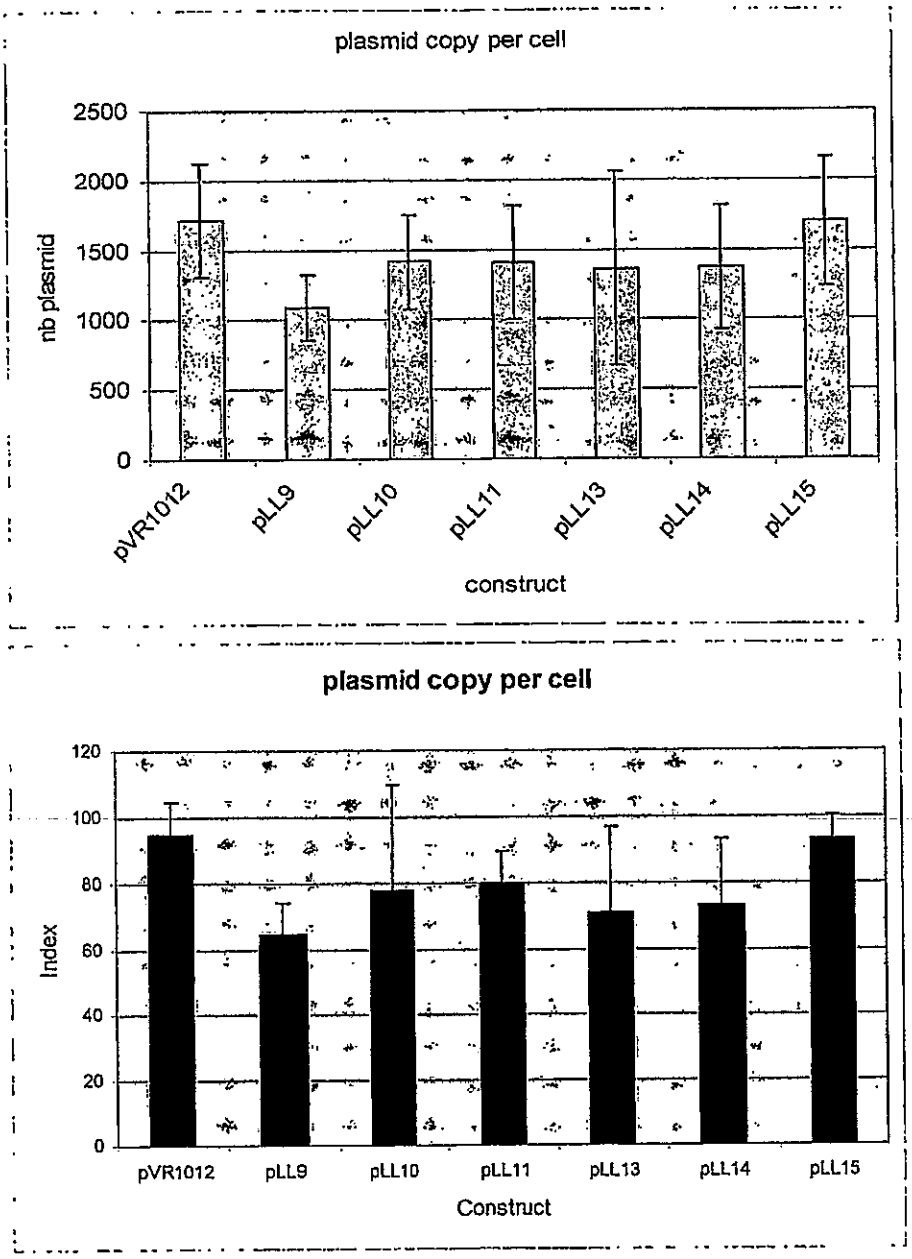

plasmid copy per cell
2500
2000
1500
1000
500
0
nb plasmid
pVR1012
pLL9
pLL10
pLL11
pLL13
pLL14
pLL15
construct
plasmid copy per cell
120
100
80
60
40
20
0
Index
pVR1012
pLL9
pLL10
pLL11
pLL13
pLL14
pLL15
Construct

FIG. 17

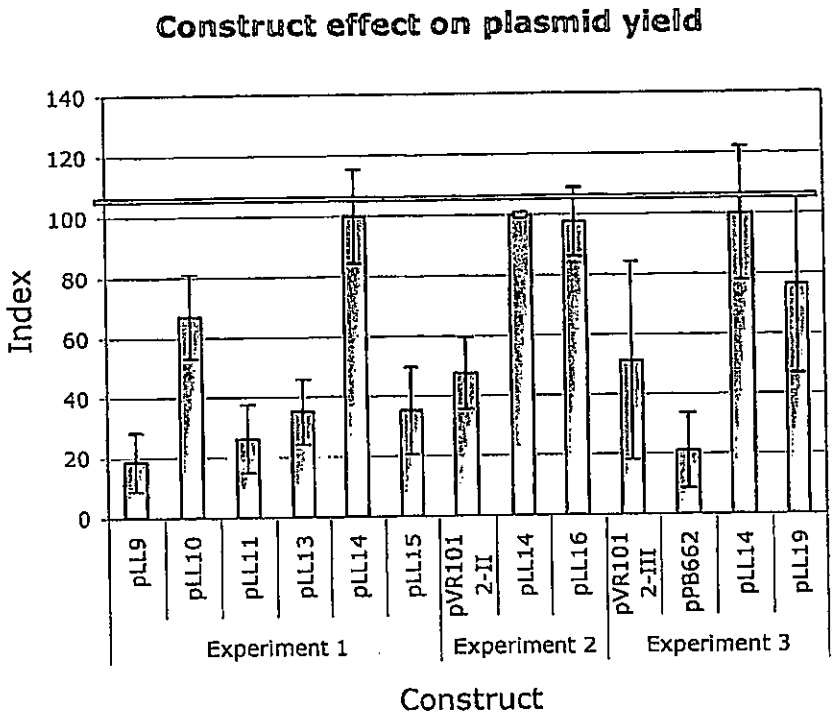

Construct effect on plasmid yield
Index
140
120
100
80
60
40
20
0
pLL9
pLL10
pLL11
pLL13
pLL14
pLL15
pVR101 2-II
pLL14
pLL16
pVR101 2-III
pPB662
pLL14
pLL19
Experiment 1
Experiment 2
Experiment 3
Construct

FIG. 18

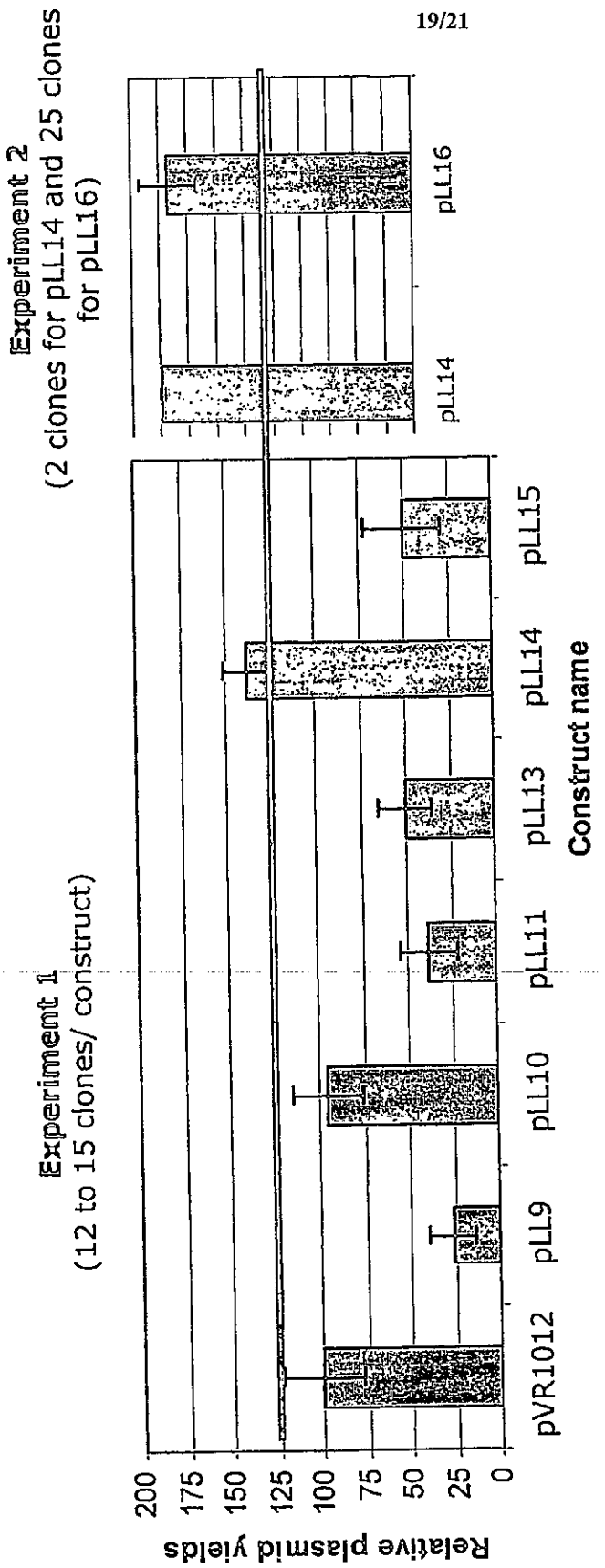
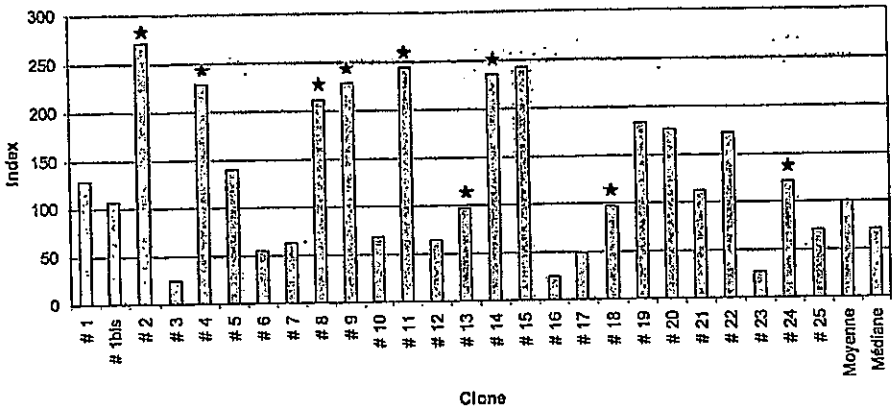
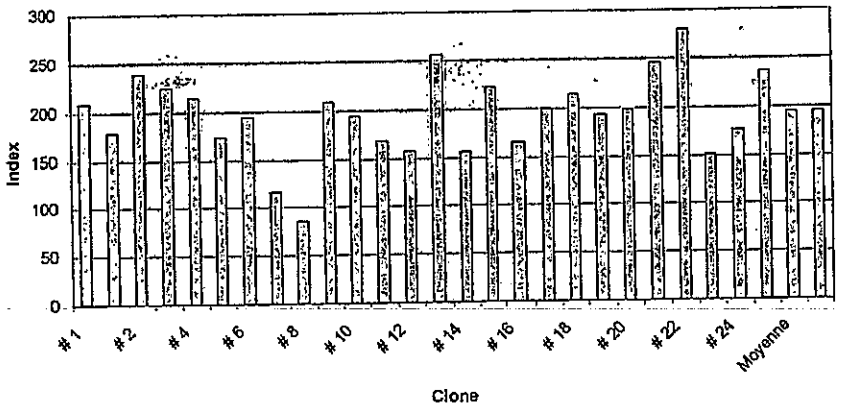

Experiment 1
(12 to 15 clones/ construct)
Experiment 2
(2 clones for pLL14 and 25 clones for pLL16)
Relative plasmid yields
200
175
150
125
100
75
50
25
0
pVR1012
pLL9
pLL10
pLL11
pLL13
pLL14
pLL15
pLL14
pLL16
Construct name

FIG. 19

pVR1012



pLL14



pLL19

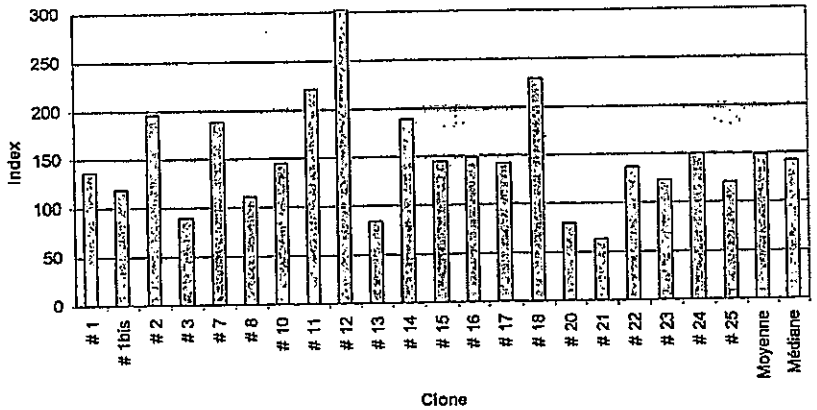


FIG. 20

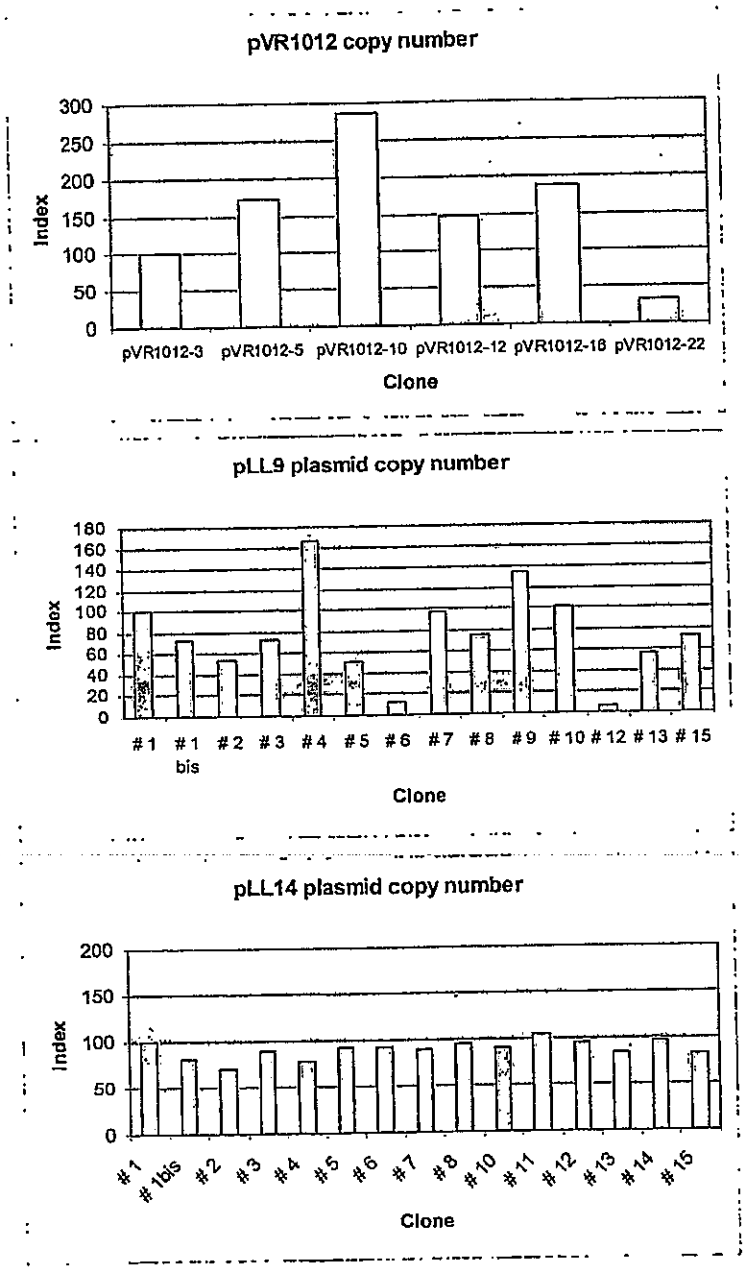

pVR1012 copy number
Index
300
250
200
150
100
50
0
pVR1012-3
pVR1012-5
pVR1012-10
pVR1012-12
pVR1012-16
pVR1012-22
Clone
pLL9 plasmid copy number
Index
180
160
140
120
100
80
60
40
20
0
#1
#1 bis
#2
#3
#4
#5
#6
#7
#8
#9
#10
#12
#13
#15
Clone
pLL14 plasmid copy number
Index
200
150
100
50
0
#1
#1bis
#2
#3
#4
#5
#6
#7
#8
#10
#11
#12
#13
#14
#15
Clone

FIG. 20