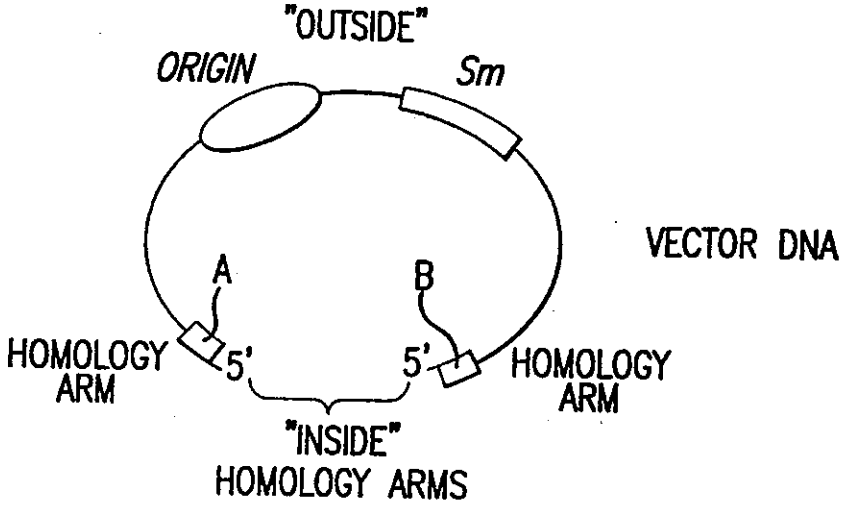


FIG.1A

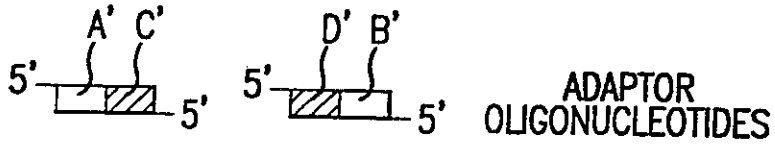


FIG.1B

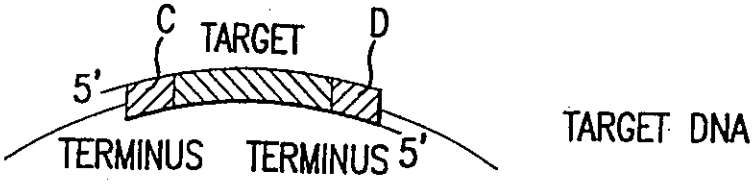


FIG.1C

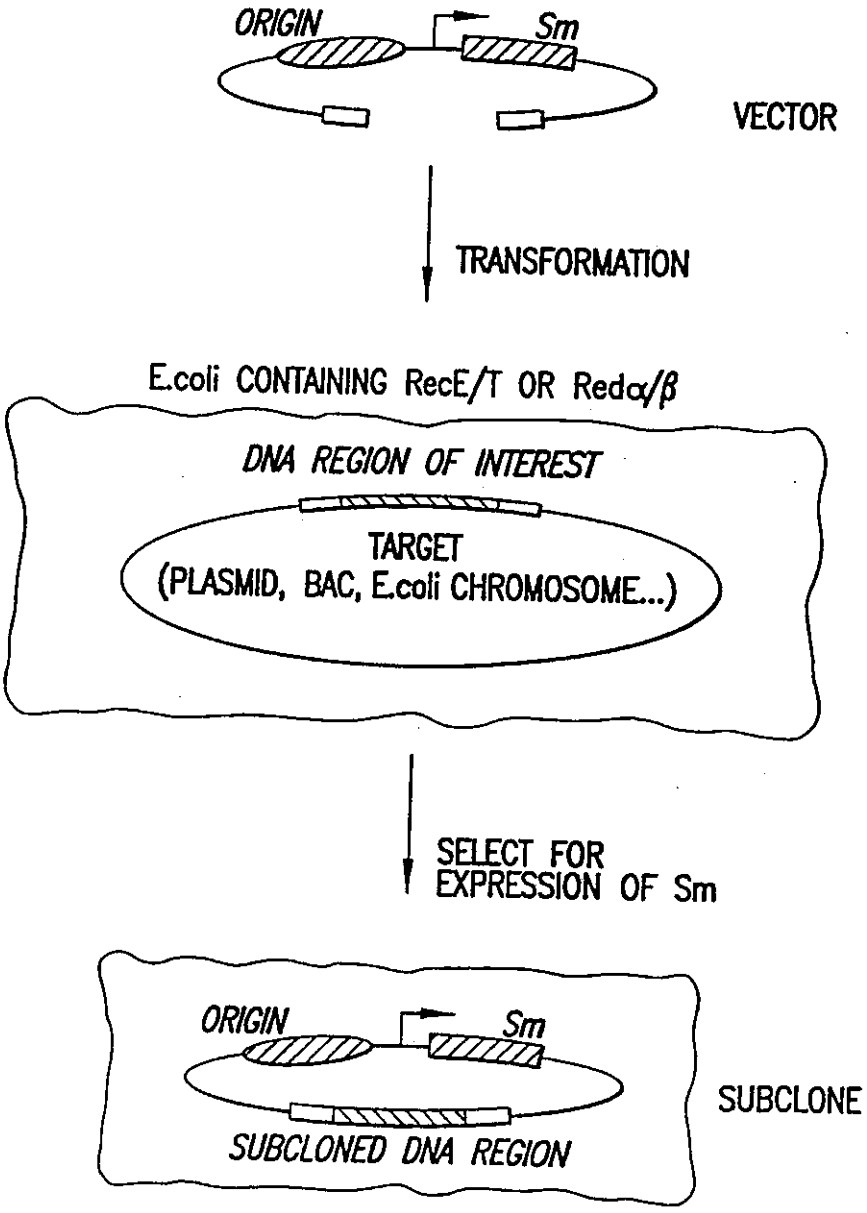

ORIGIN
Sm
VECTOR
TRANSFORMATION
E.coli CONTAINING RecE/T OR Redα/β
DNA REGION OF INTEREST
TARGET
(PLASMID, BAC, E.coli CHROMOSOME...)
SELECT FOR
EXPRESSION OF Sm
ORIGIN
Sm
SUBCLONE
SUBCLONED DNA REGION

FIG.2

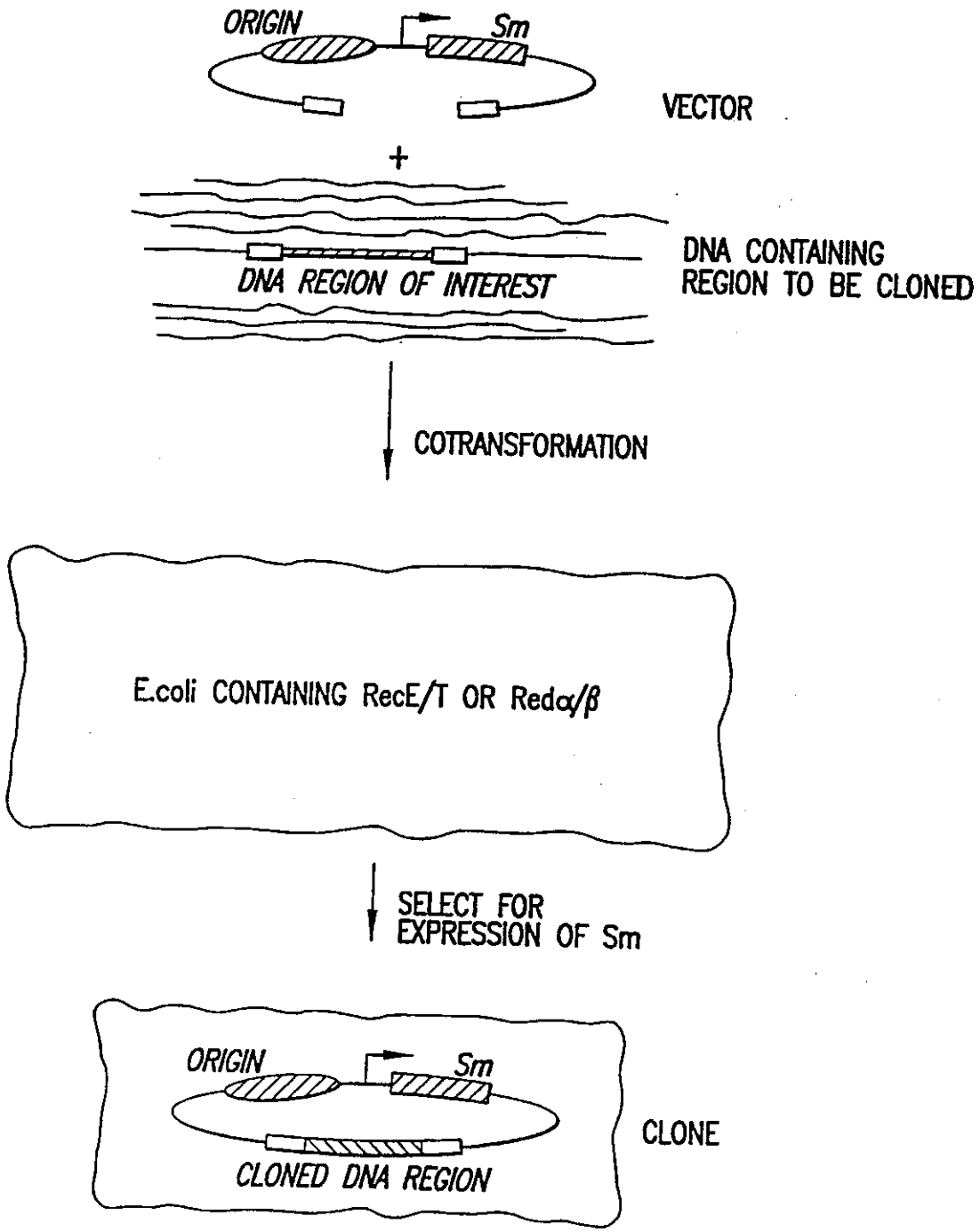

ORIGIN
Sm
VECTOR
+
DNA REGION OF INTEREST
DNA CONTAINING
REGION TO BE CLONED
COTRANSFORMATION
E.coli CONTAINING RecE/T OR Redα/β
SELECT FOR
EXPRESSION OF Sm
ORIGIN
Sm
CLONED DNA REGION
CLONE

FIG.3

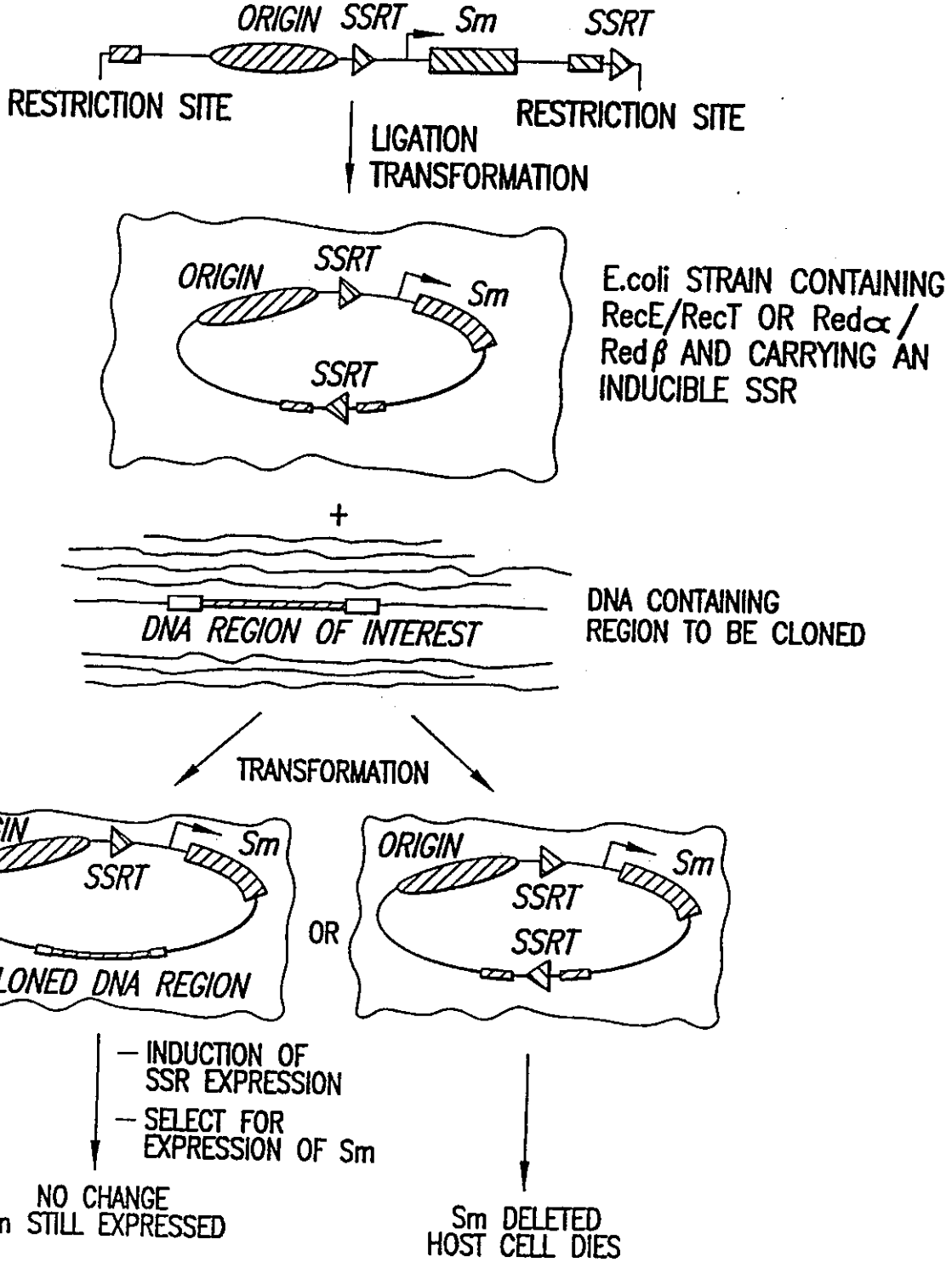

ORIGIN
SSRT
Sm
SSRT
RESTRICTION SITE
RESTRICTION SITE
LIGATION
TRANSFORMATION
ORIGIN
SSRT
Sm
SSRT
E.coli STRAIN CONTAINING RecE/RecT OR Redα / Redβ AND CARRYING AN INDUCIBLE SSR
+
DNA REGION OF INTEREST
DNA CONTAINING REGION TO BE CLONED
TRANSFORMATION
ORIGIN
Sm
SSRT
CLONED DNA REGION
OR
ORIGIN
Sm
SSRT
SSRT
– INDUCTION OF SSR EXPRESSION
– SELECT FOR EXPRESSION OF Sm
NO CHANGE
Sm STILL EXPRESSED
Sm DELETED
HOST CELL DIES

FIG.4

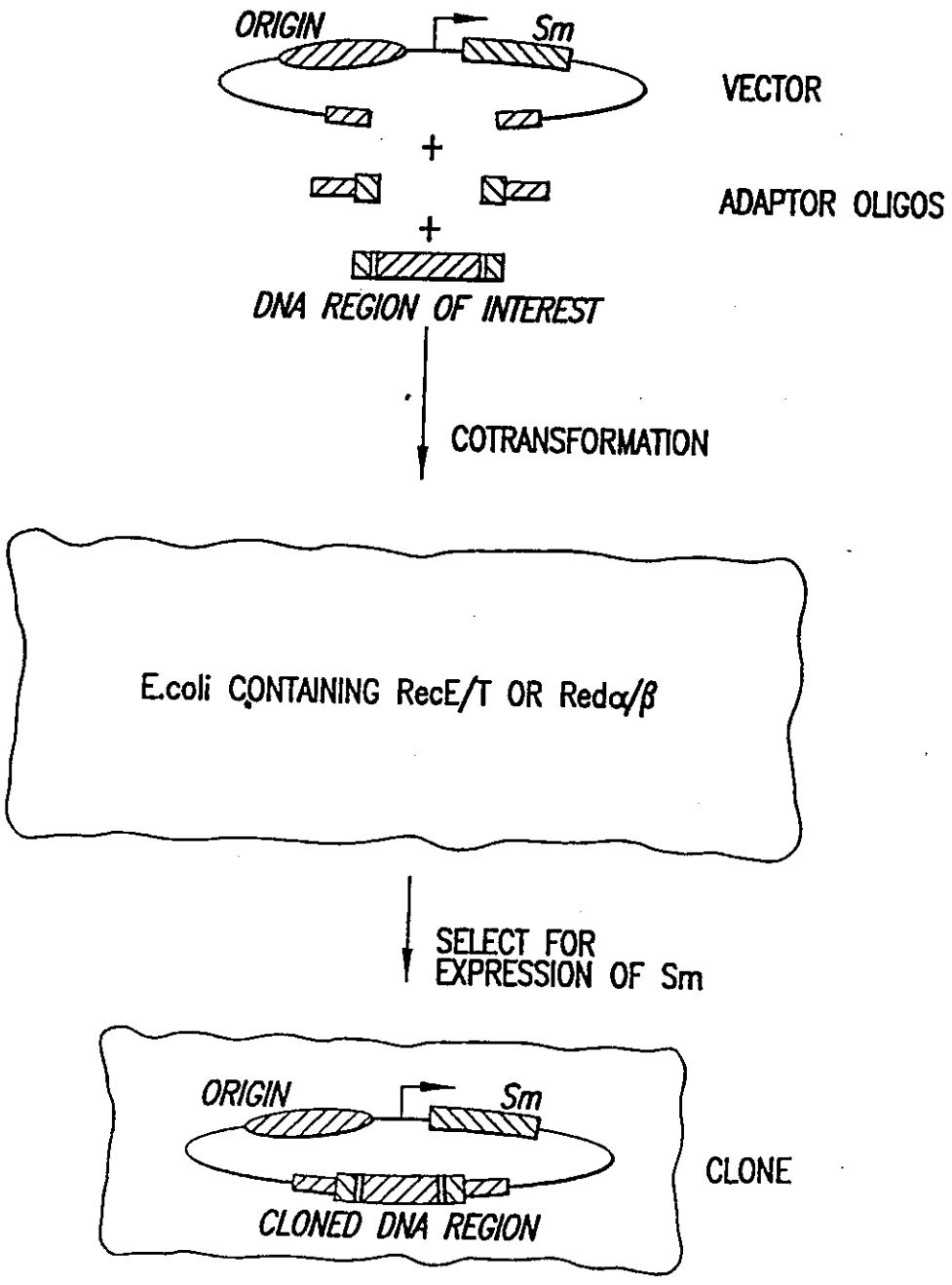

ORIGIN
Sm
VECTOR
+
ADAPTOR OLIGOS
+
DNA REGION OF INTEREST
COTRANSFORMATION
E.coli CONTAINING RecE/T OR Redα/β
SELECT FOR EXPRESSION OF Sm
ORIGIN
Sm
CLONED DNA REGION
CLONE

FIG.5

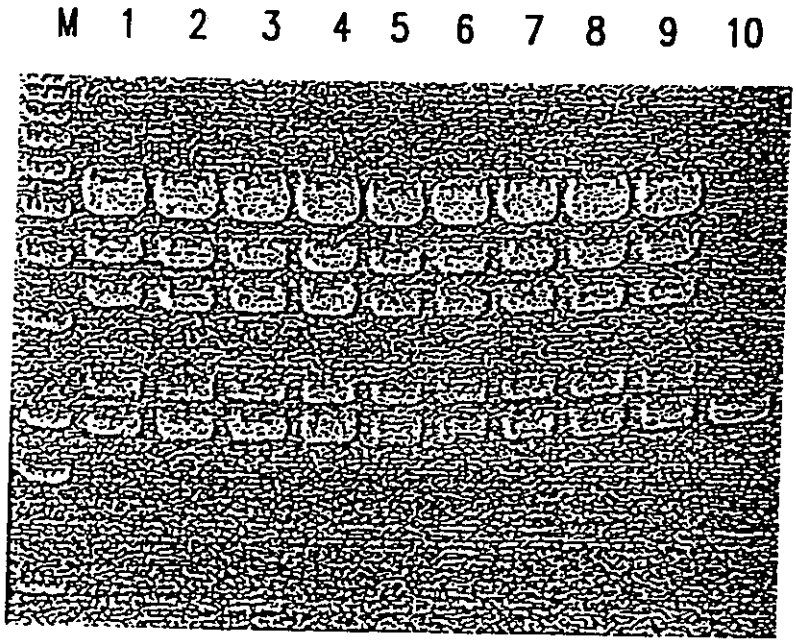


FIG.6

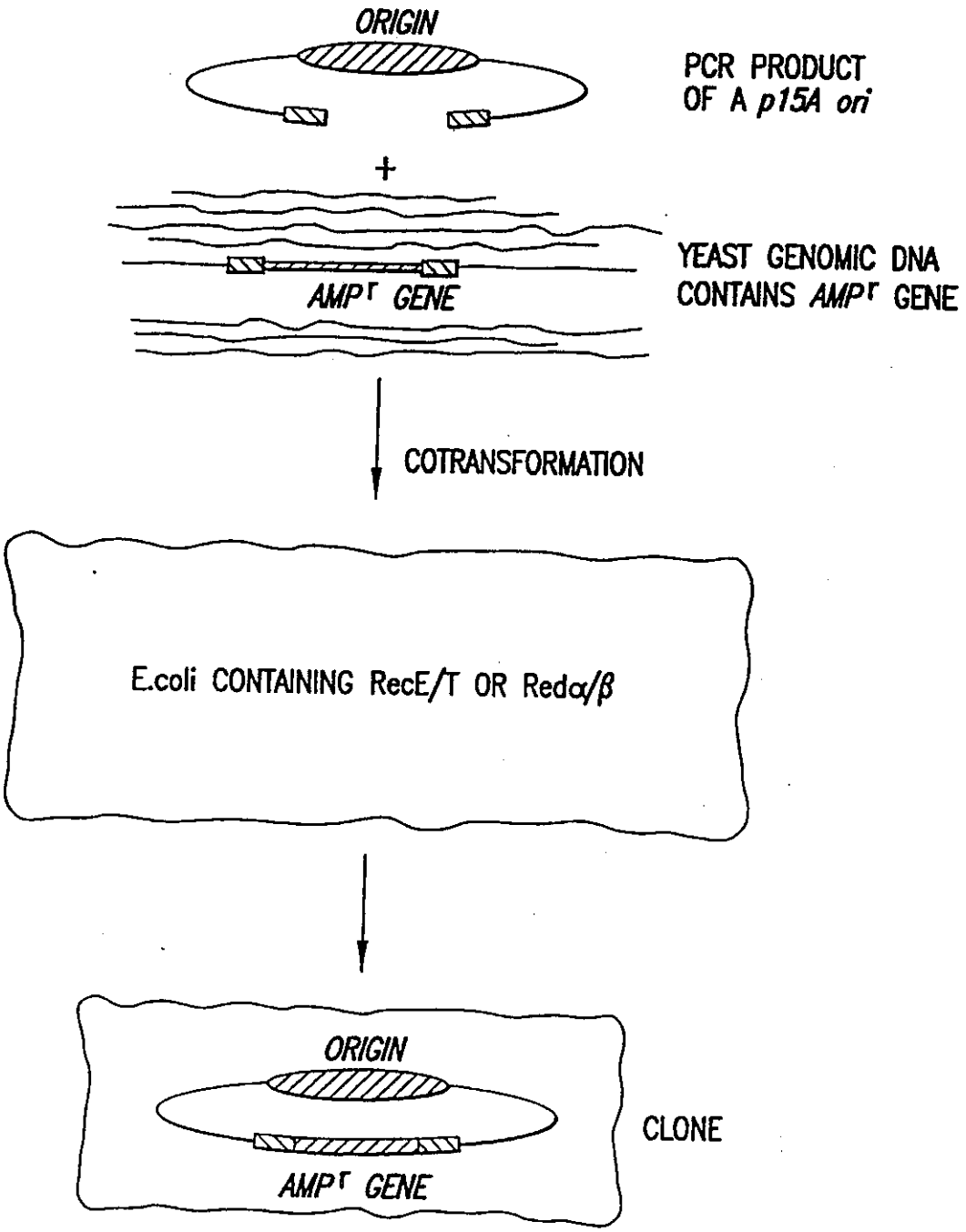

ORIGIN
PCR PRODUCT
OF A p15A ori
+
YEAST GENOMIC DNA
CONTAINS AMPr GENE
AMPr GENE
COTRANSFORMATION
E.coli CONTAINING RecE/T OR Redα/β
ORIGIN
AMPr GENE
CLONE

FIG.7A

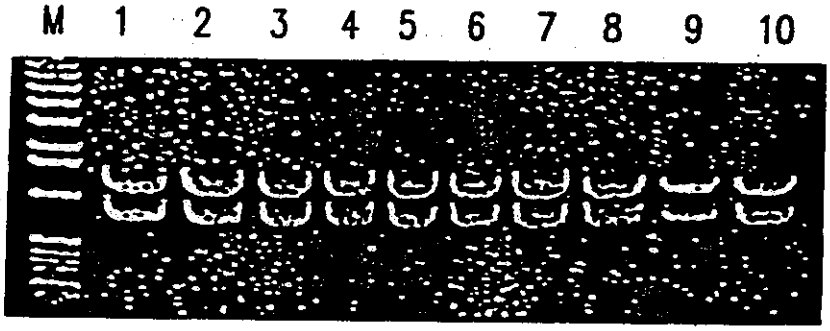


FIG.7B

EFFECT OF REPEATS AND PHOSPHORYLATION AT THE END OF THE LINEAR VECTOR

LEFT:
a, CGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTT*ACAACTTATATCGTATGGGG*
b, TGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTT*ACAACTTATATCGTATGGGG*
c, GCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTT*ACAACTTATATCGTATGGGG*
d, CAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTT*ACAACTTATATCGTATGGGG*
e, CGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTT*ACAACTTATATCGTATGGGG*

RIGHT:
x, GCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGC*TTACGCCCCGCCCTGCCAC*

FIG.8A

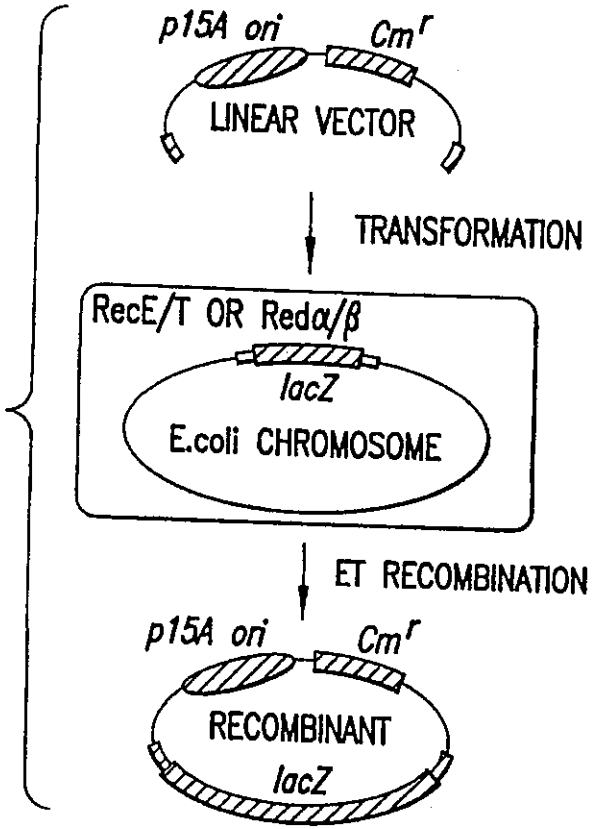


FIG.8B

COMBINATION OF PRIMERS	x–a	x–b	x–c	x–d	x–e	phosphorylated x–a
RECOMBINANTS	548	562	827	2142	5780	542
EFFICIENCY	100%	100%	67%	22%	5.6%	100%

FIG.8C

SUBSTITUTE SHEET (RULE 26)

9 FEB 2

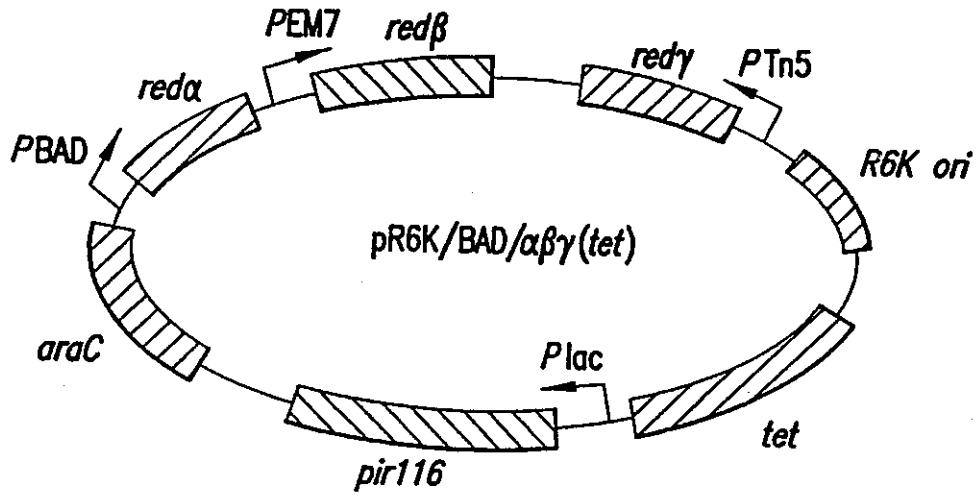


FIG.9A

ET SUBCLONING FROM AF4 BAC

CONSTRUCT	pBAD–αβγ/tet (ColE1 ori)		pR6K/BAD/αβγ	
EXPERIMENT	1	2	1	2
RECOMBINANTS	385	347	~1,200	~1,300

FIG.9B

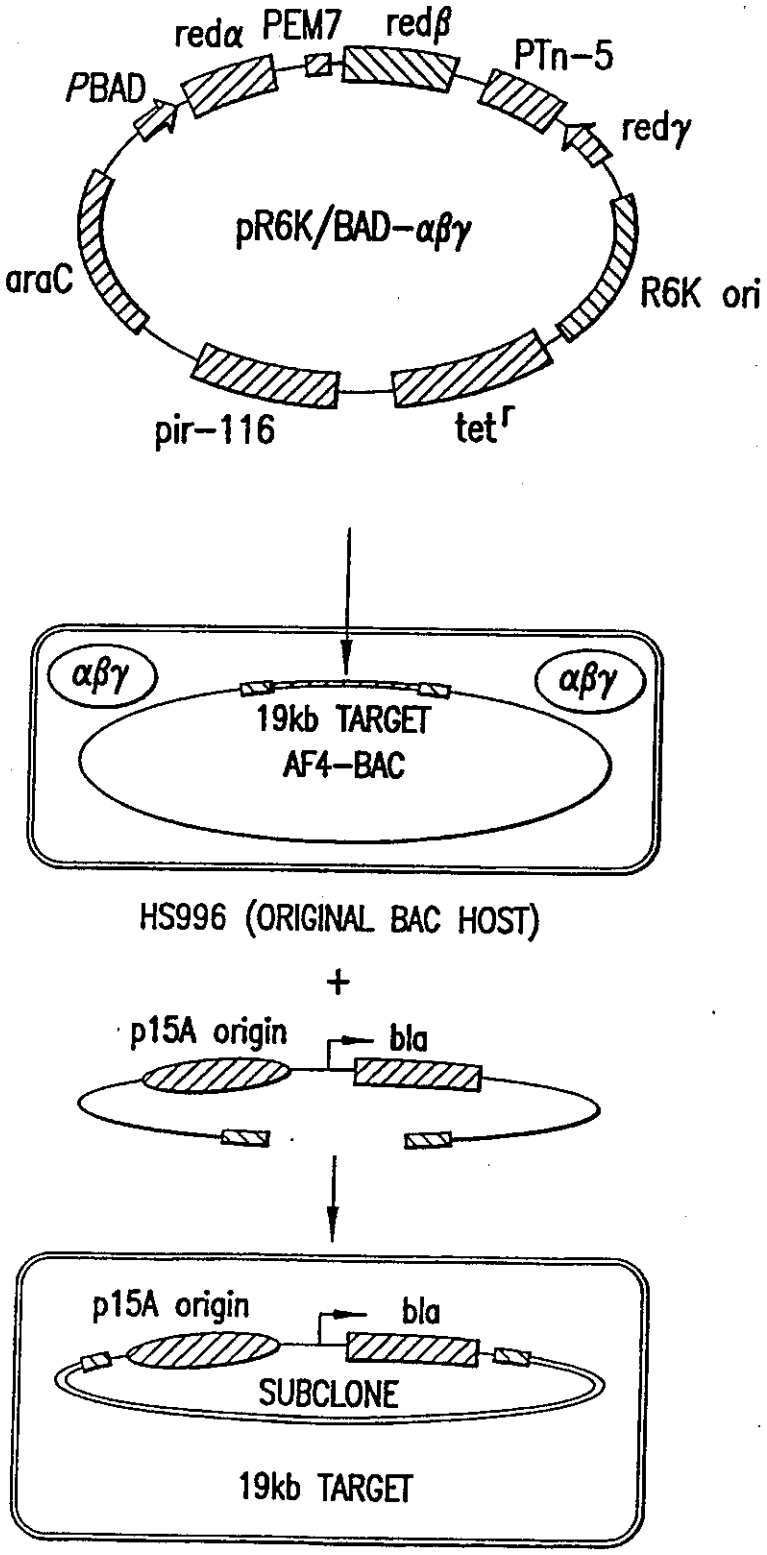

redα
PEM7
redβ
PTn-5
PBAD
redγ
pR6K/BAD-αβγ
araC
R6K ori
pir-116
tet^r
αβγ
αβγ
19kb TARGET
AF4-BAC
HS996 (ORIGINAL BAC HOST)
+
p15A origin
bla
p15A origin
bla
SUBCLONE
19kb TARGET

FIG.10A

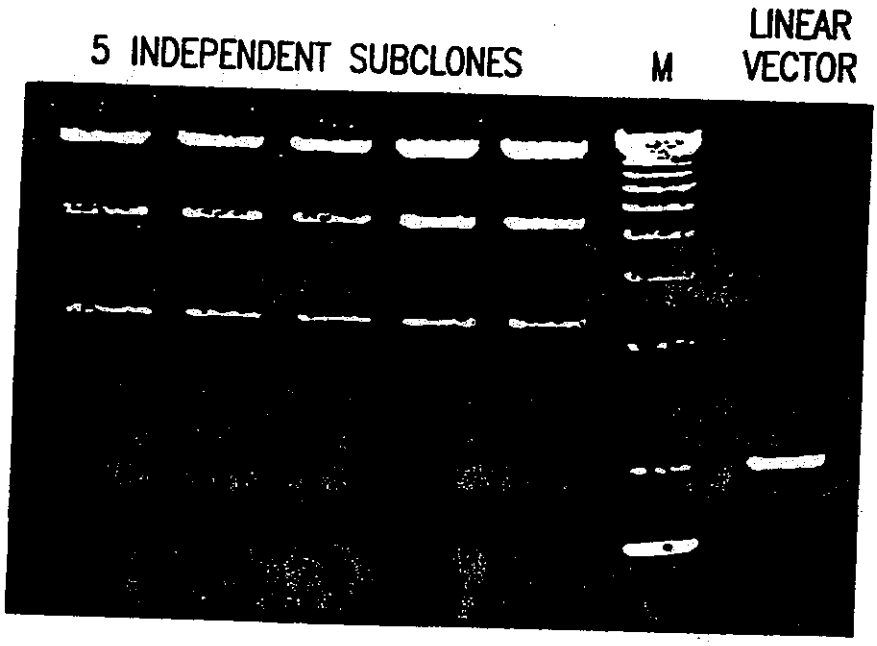


FIG.10B

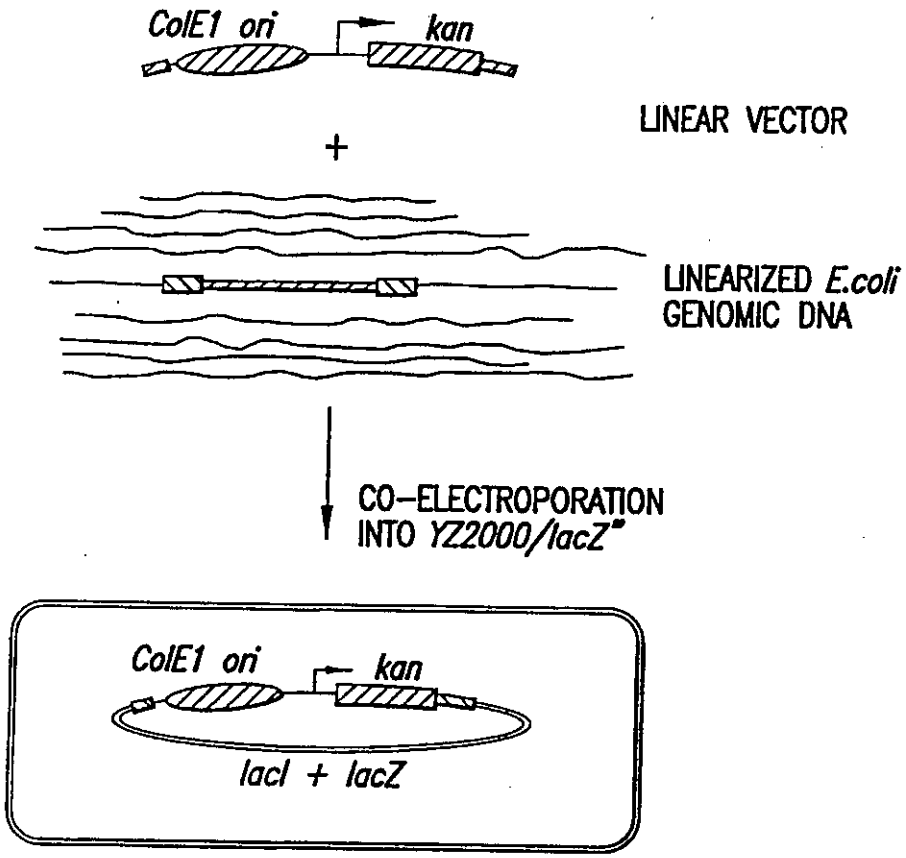

ColE1 ori
kan
LINEAR VECTOR
+
LINEARIZED E.coli
GENOMIC DNA
CO-ELECTROPORATION
INTO YZ2000/lacZ⁻
ColE1 ori
kan
lacI + lacZ

FIG.11A

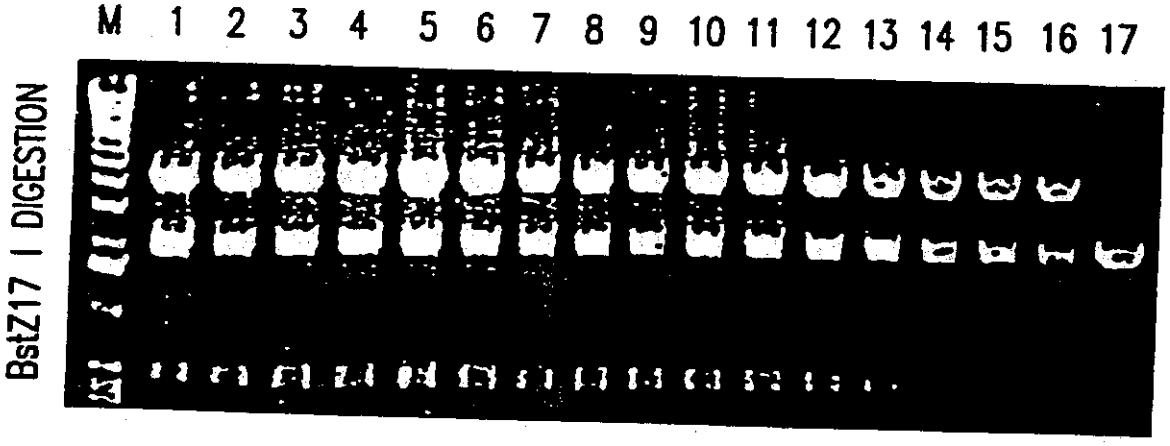


FIG.11B

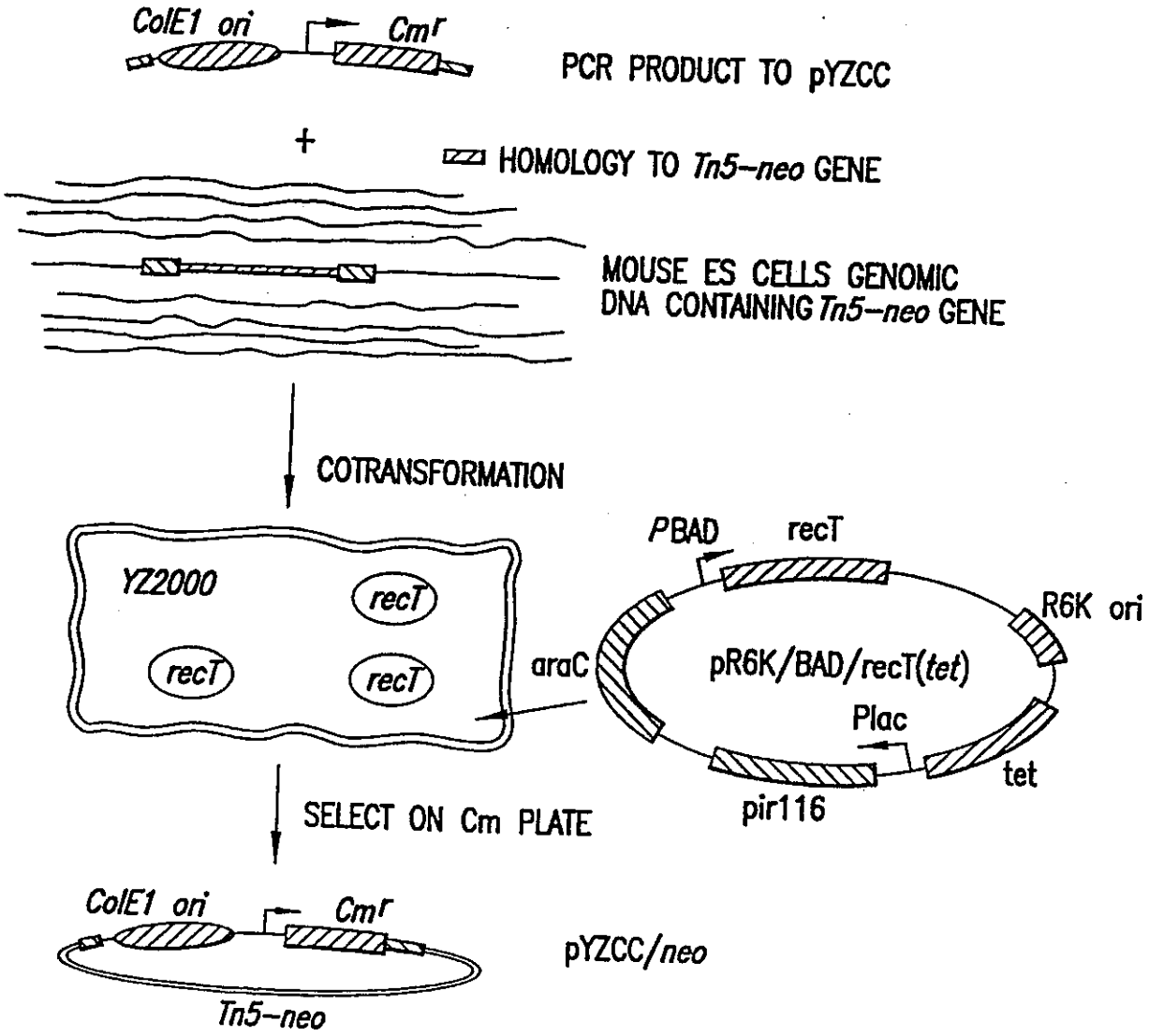

ColE1 ori
Cmr
PCR PRODUCT TO pYZCC
+
HOMOLOGY TO Tn5–neo GENE
MOUSE ES CELLS GENOMIC
DNA CONTAINING Tn5–neo GENE
COTRANSFORMATION
YZ2000
recT
recT
recT
PBAD
recT
R6K ori
araC
pR6K/BAD/recT(tet)
Plac
tet
pir116
SELECT ON Cm PLATE
ColE1 ori
Cmr
pYZCC/neo
Tn5–neo

FIG.12A

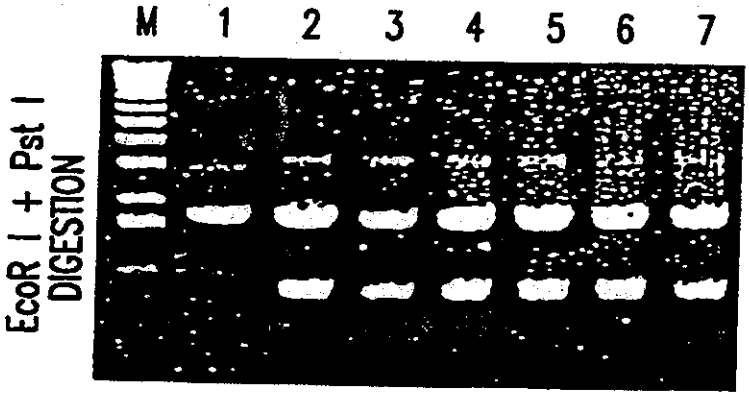


FIG.12B

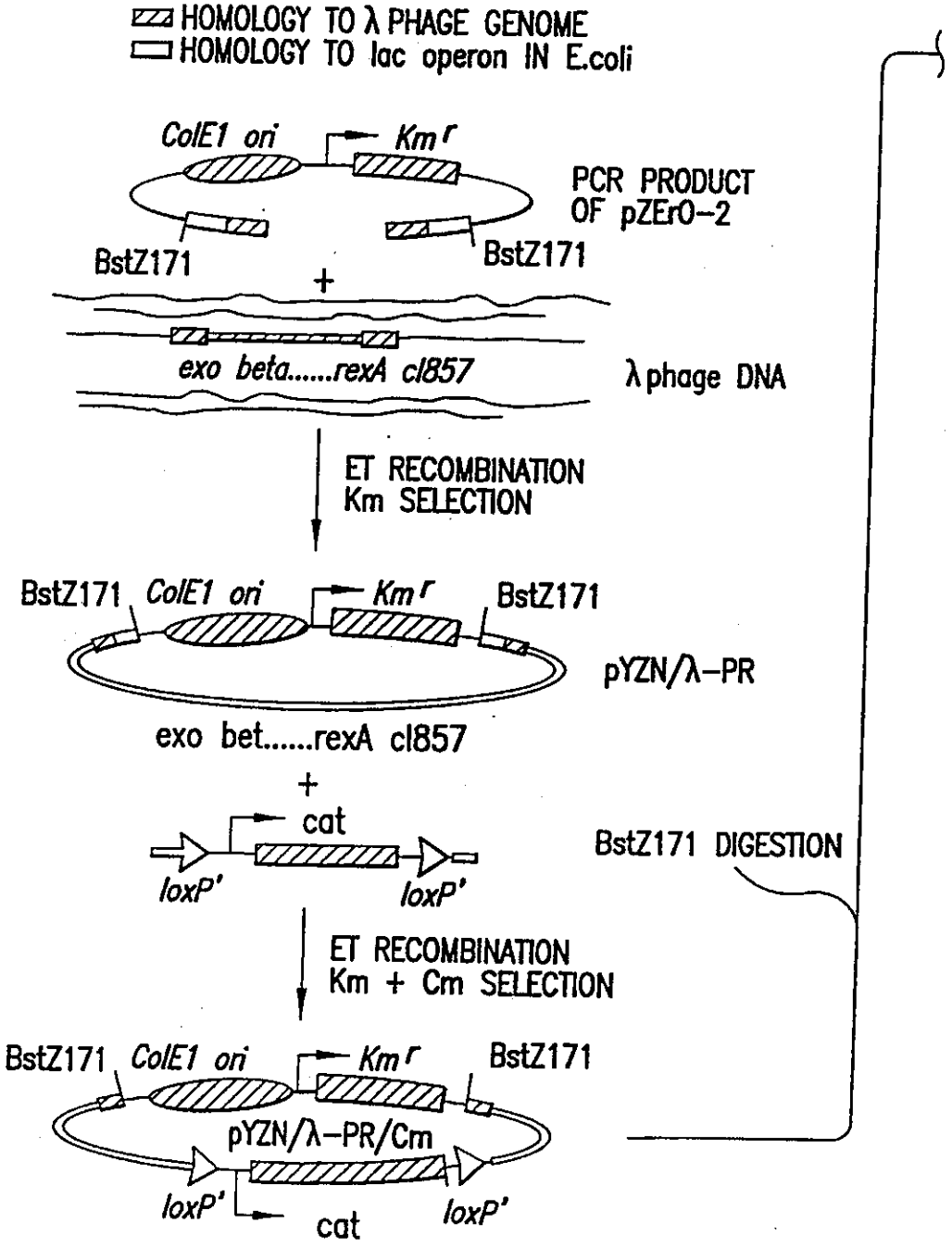

HOMOLOGY TO λ PHAGE GENOME
HOMOLOGY TO lac operon IN E.coli
ColE1 ori
Km r
PCR PRODUCT OF pZErO-2
BstZ171
BstZ171
+
exo beta.....rexA cI857
λ phage DNA
ET RECOMBINATION
Km SELECTION
BstZ171
ColE1 ori
Km r
BstZ171
pYZN/λ-PR
exo bet.....rexA cI857
+
cat
loxP'
loxP'
BstZ171 DIGESTION
ET RECOMBINATION
Km + Cm SELECTION
BstZ171
ColE1 ori
Km r
BstZ171
pYZN/λ-PR/Cm
loxP'
cat
loxP'

FIG.13A

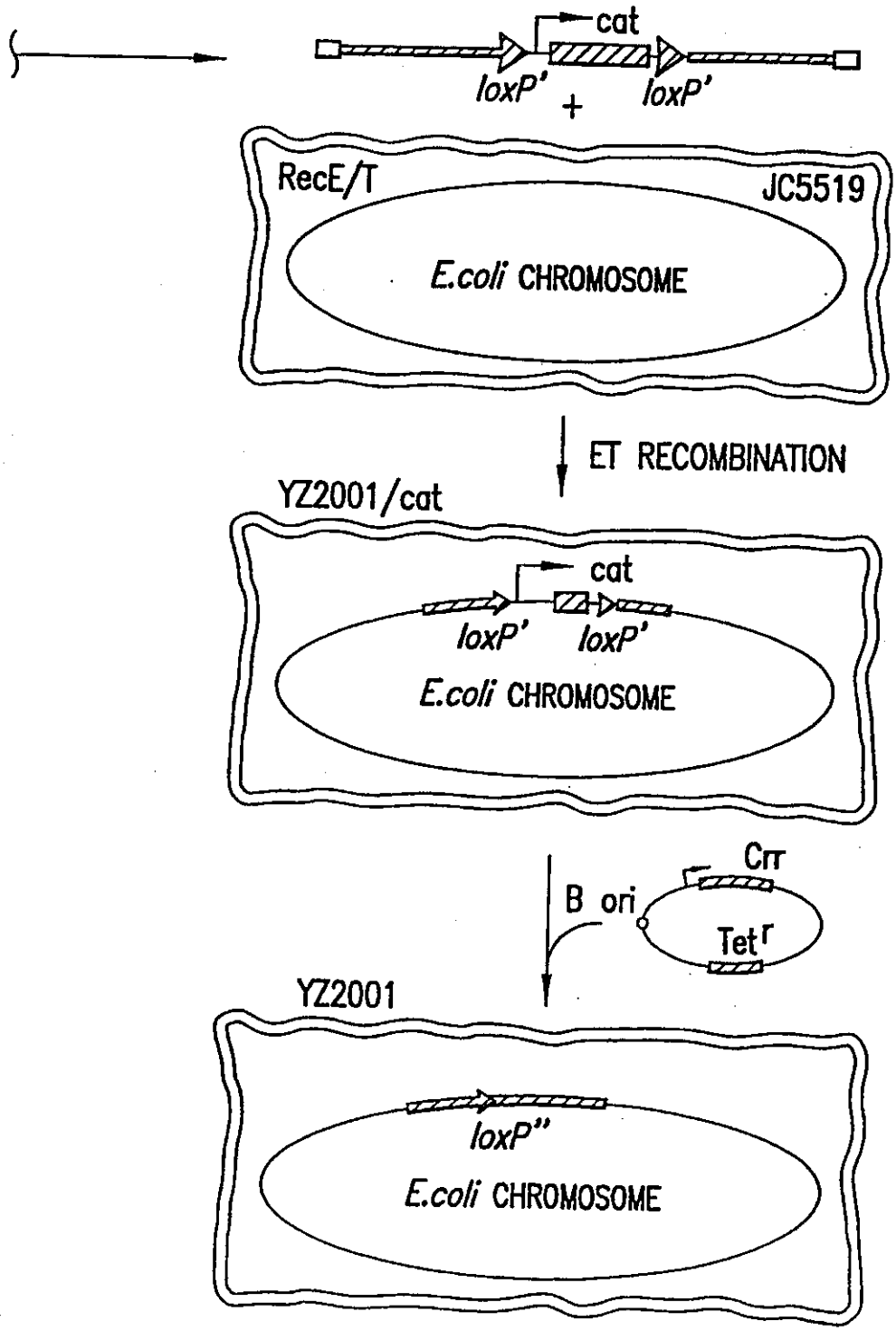

cat
loxP'
+
loxP'
RecE/T
JC5519
E.coli CHROMOSOME
ET RECOMBINATION
YZ2001/cat
cat
loxP'
loxP'
E.coli CHROMOSOME
B ori
Cm
Tet
YZ2001
loxP"
E.coli CHROMOSOME

FIG.13B