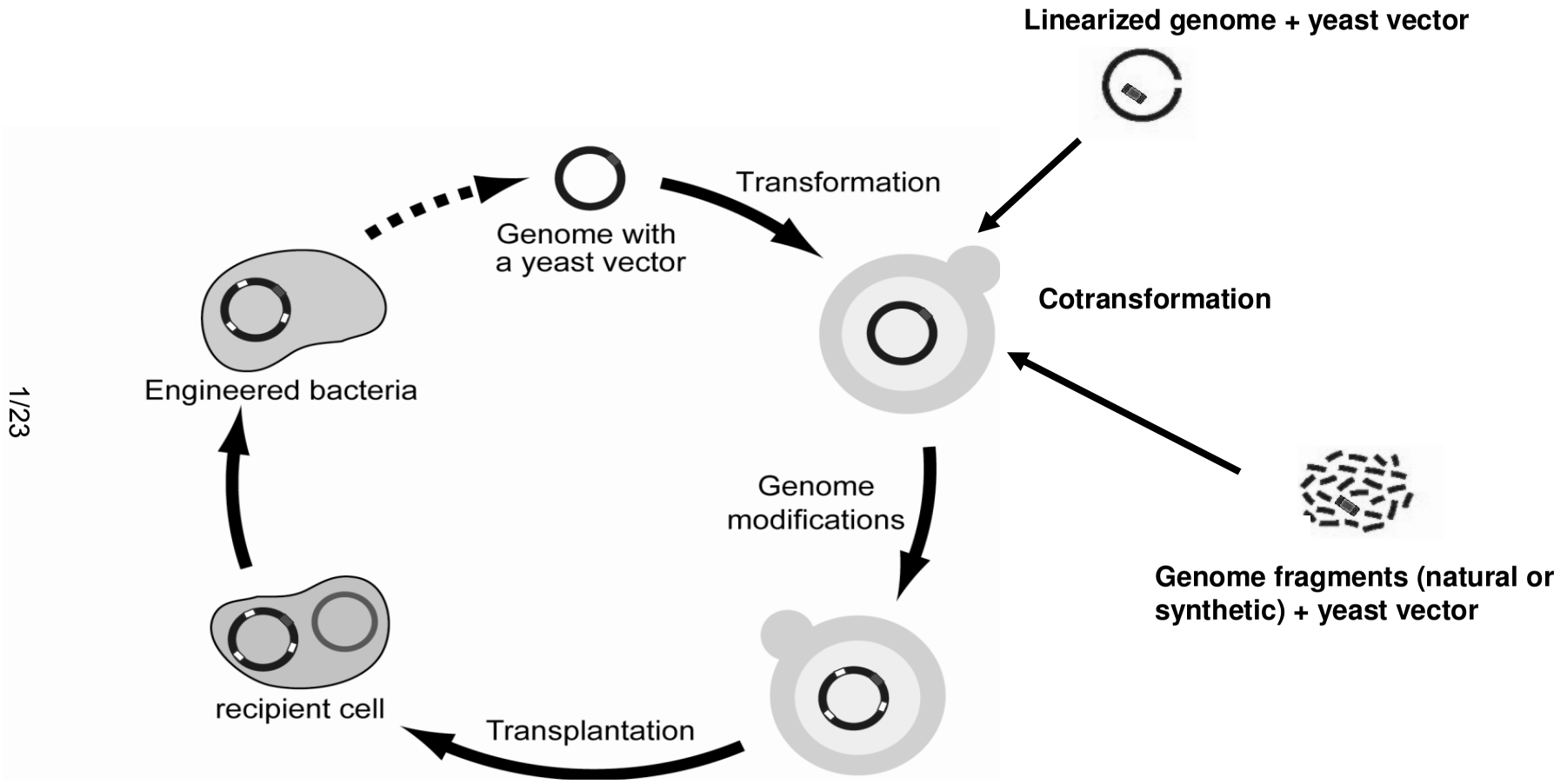

Linearized genome + yeast vector
Transformation
Genome with
a yeast vector
Cotransformation
Engineered bacteria
Genome
modifications
Genome fragments (natural or
synthetic) + yeast vector
recipient cell
Transplantation

FIG. 1

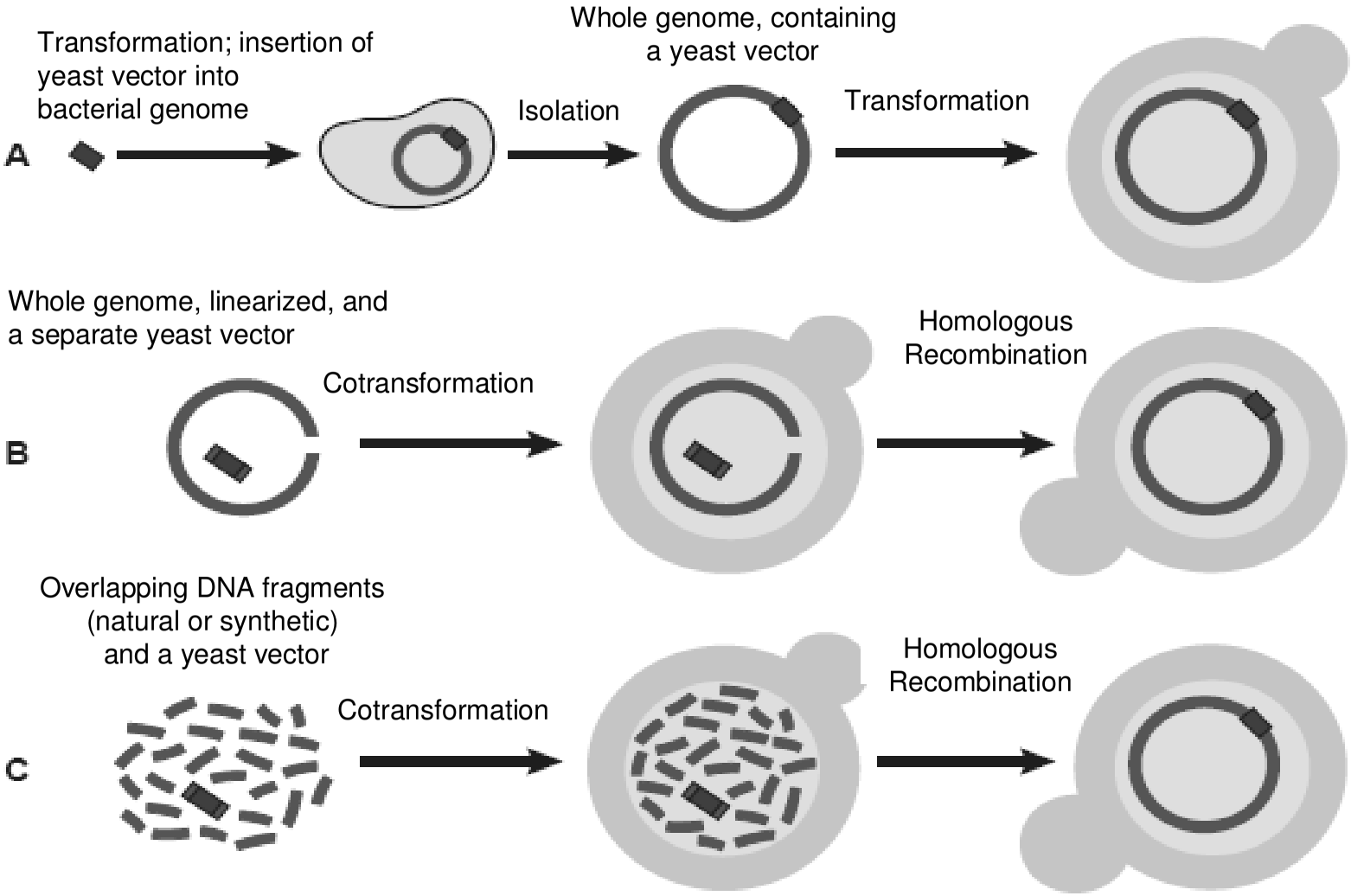

Transformation; insertion of
yeast vector into
bacterial genome
A
Isolation
Whole genome, containing
a yeast vector
Transformation
Whole genome, linearized, and
a separate yeast vector
B
Cotransformation
Homologous
Recombination
Overlapping DNA fragments
(natural or synthetic)
and a yeast vector
C
Cotransformation
Homologous
Recombination

FIG. 2

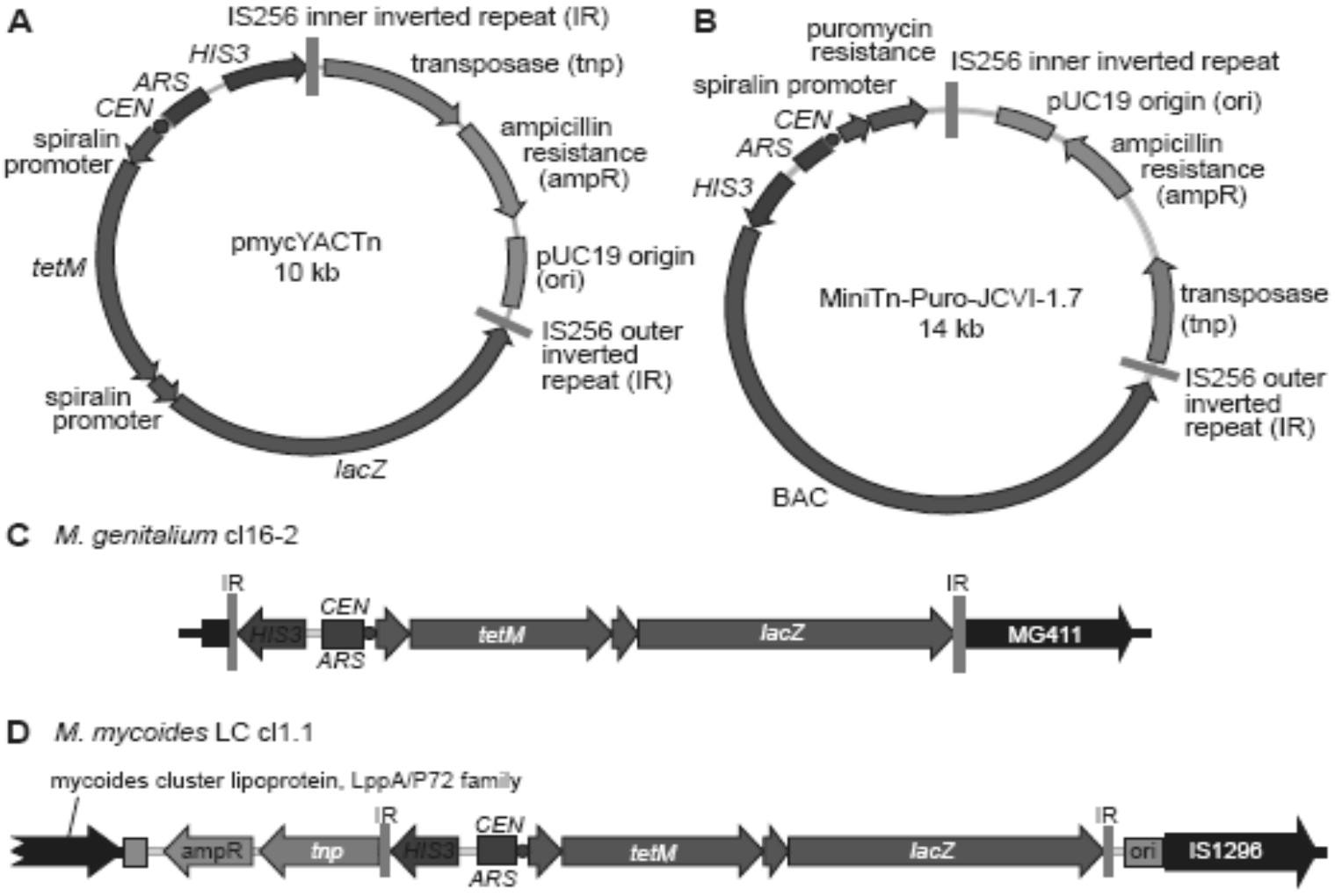

A
IS256 inner inverted repeat (IR)
HIS3
ARS
CEN
spiralin promoter
transposase (tnp)
ampicillin resistance (ampR)
pmycYACTn 10 kb
pUC19 origin (ori)
tetM
IS256 outer inverted repeat (IR)
spiralin promoter
lacZ
B
puromycin resistance
IS256 inner inverted repeat
spiralin promoter
pUC19 origin (ori)
CEN
ARS
ampicillin resistance (ampR)
HIS3
MiniTn-Puro-JCVI-1.7 14 kb
transposase (tnp)
IS256 outer inverted repeat (IR)
BAC
C M. genitalium cI16-2
IR
CEN
HIS3
ARS
tetM
lacZ
IR
MG411
D M. mycoides LC cI1.1
mycoides cluster lipoprotein, LppA/P72 family
IR
CEN
ampR
tnp
HIS3
ARS
tetM
lacZ
IR
ori
IS1296

FIG. 3

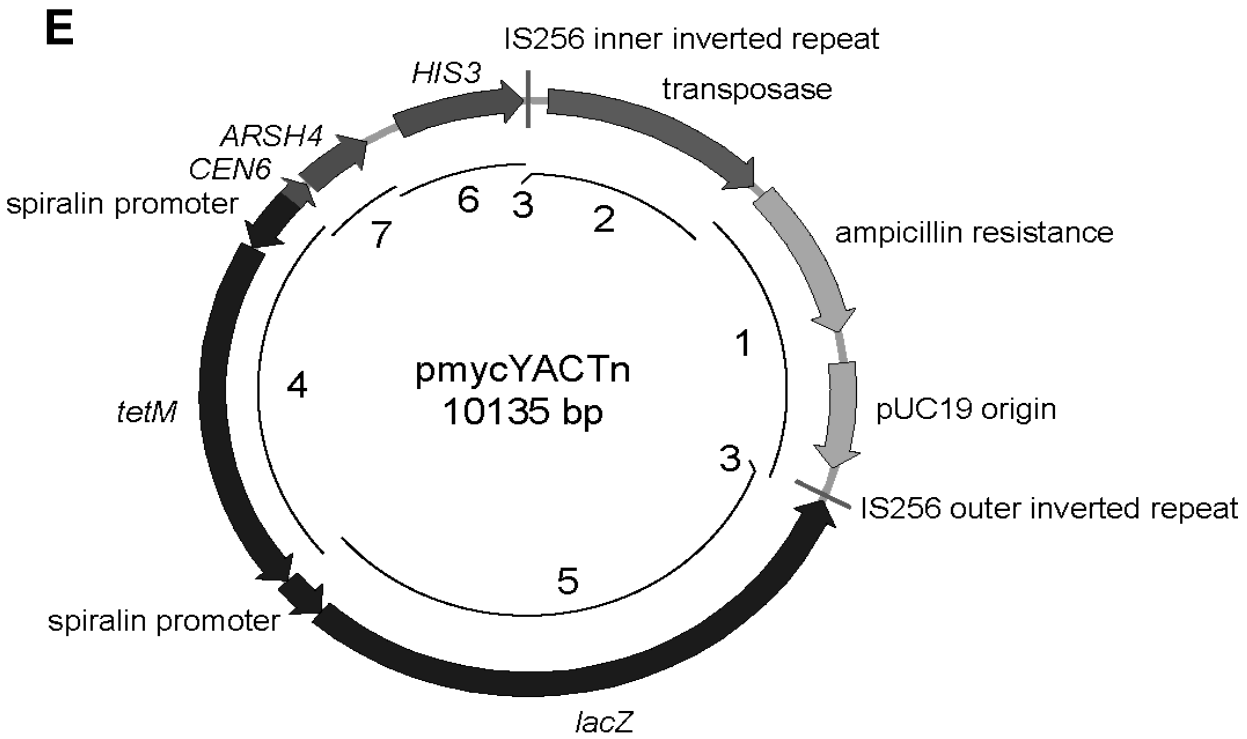
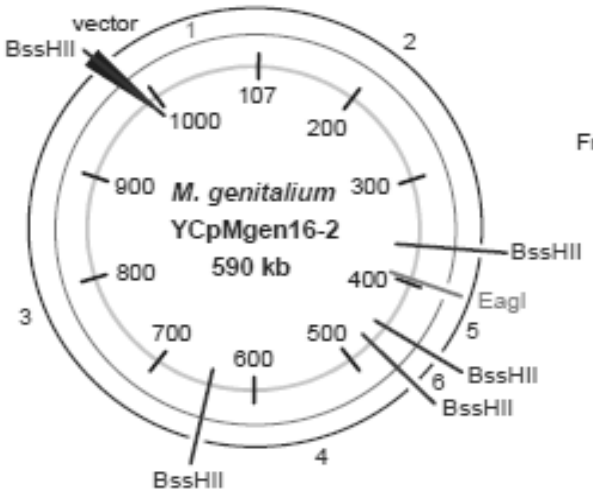

E
IS256 inner inverted repeat
HIS3
transposase
ARSH4
CEN6
spiralin promoter
ampicillin resistance
pmycYACTn
10135 bp
tetM
pUC19 origin
IS256 outer inverted repeat
spiralin promoter
lacZ
F
YCpMpn028
MPN028
IR
puromycin resistance
IR
CEN
BAC
HIS3
ARS

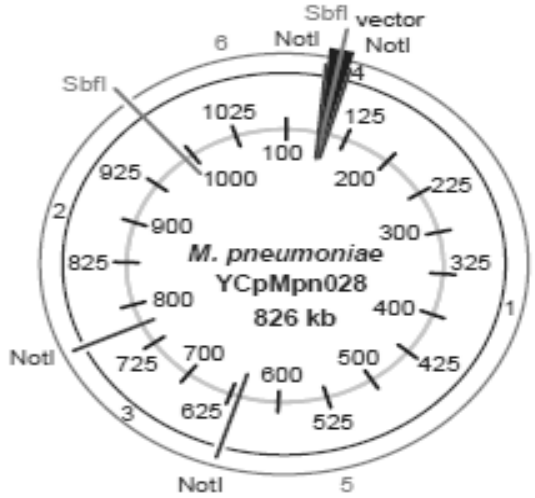
FIG. 3

A



Fragment	Size (kb)
1	590
2	226
3	204
4	97
5	51
6	12

B



Fragment	Size (kb)
1	417
2	294
3	105
4	10
5	707
6	119

FIG. 4

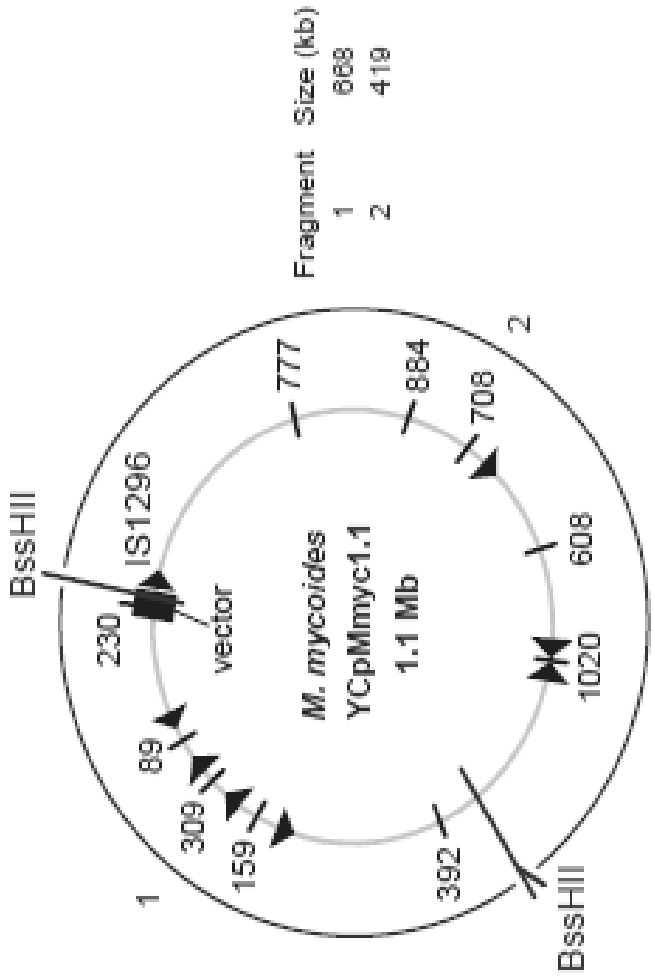

Fragment Size (kb)
1 668
2 419
BssHII
IS1296
230
vector
89
309
159
1
777
884
708
608
1020
392
2
BssHII
M. mycoides
YCpMmyc1.1
1.1 Mb

FIG. 5

FIG. 6

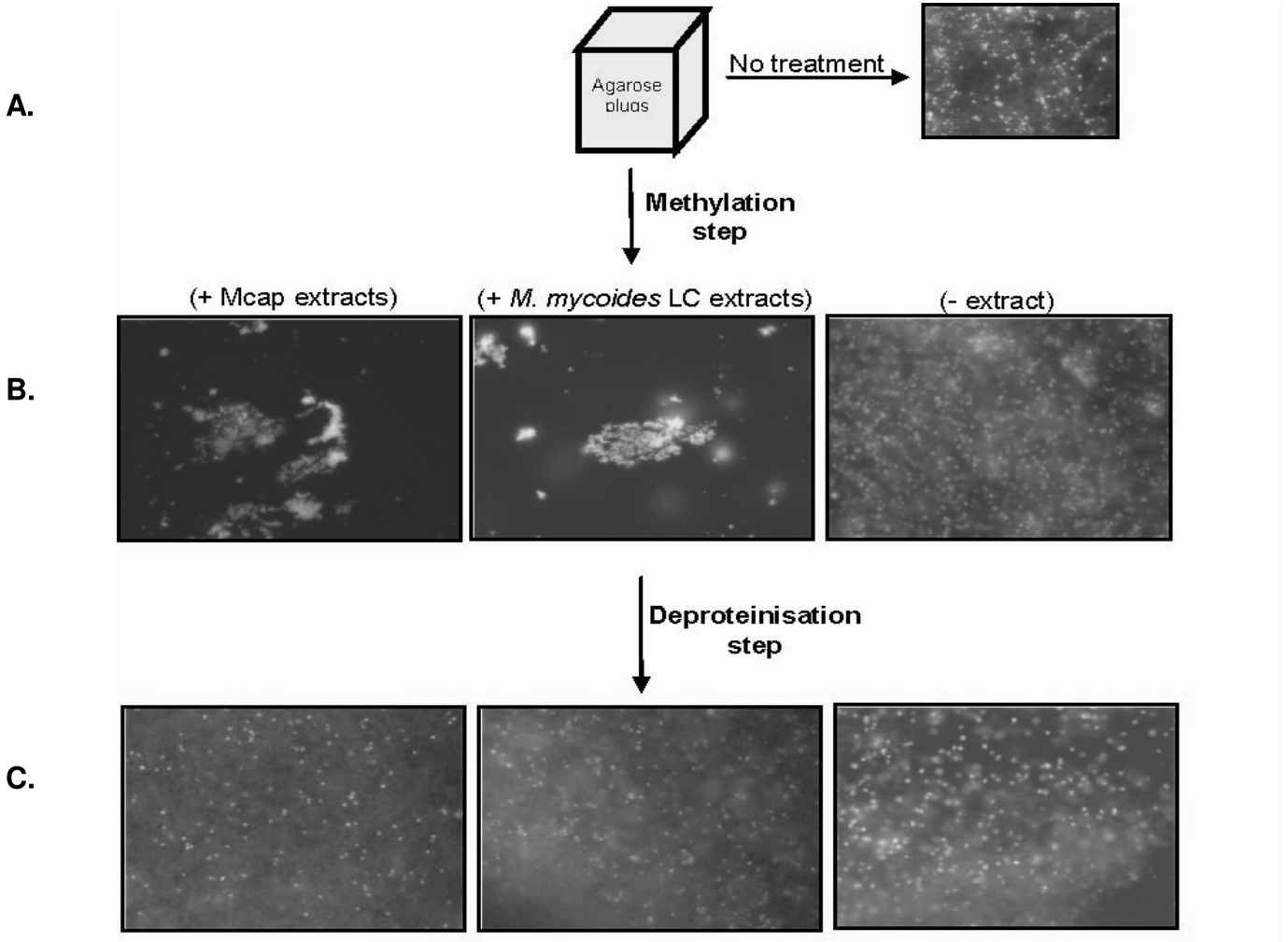

A.
Agarose plugs
No treatment
Methylation step
B.
(+ Mcap extracts)
(+ M. mycoides LC extracts)
(- extract)
Deproteinisation step
C.

FIG. 7

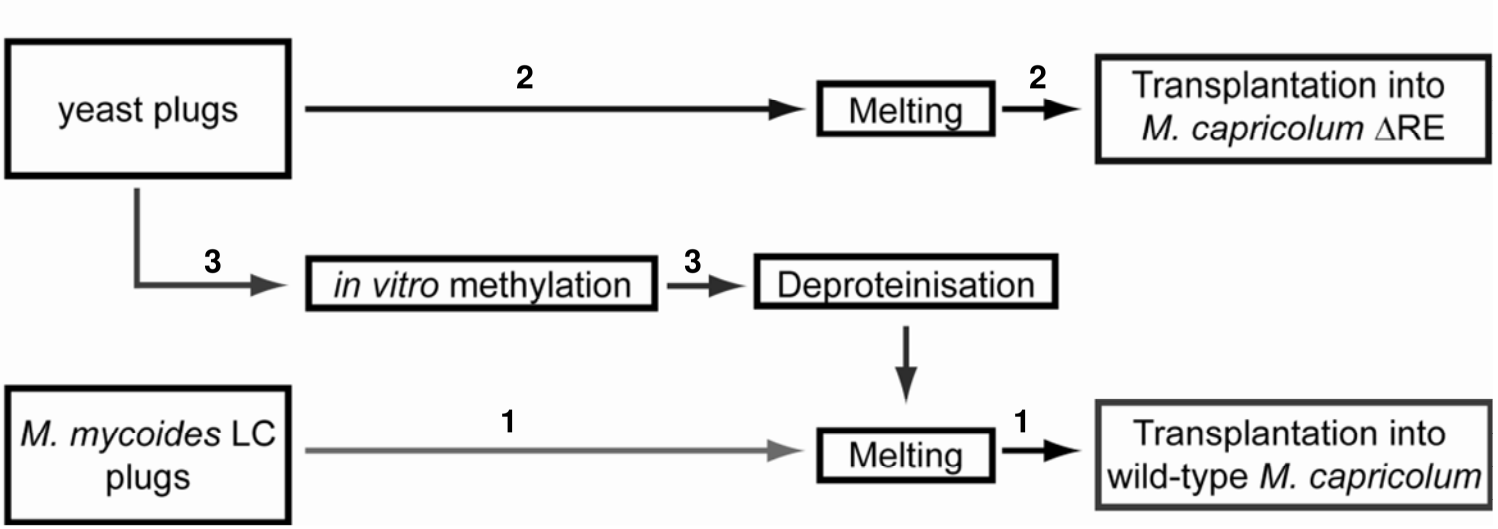


FIG. 8
Whole Genome Transplantation Methods

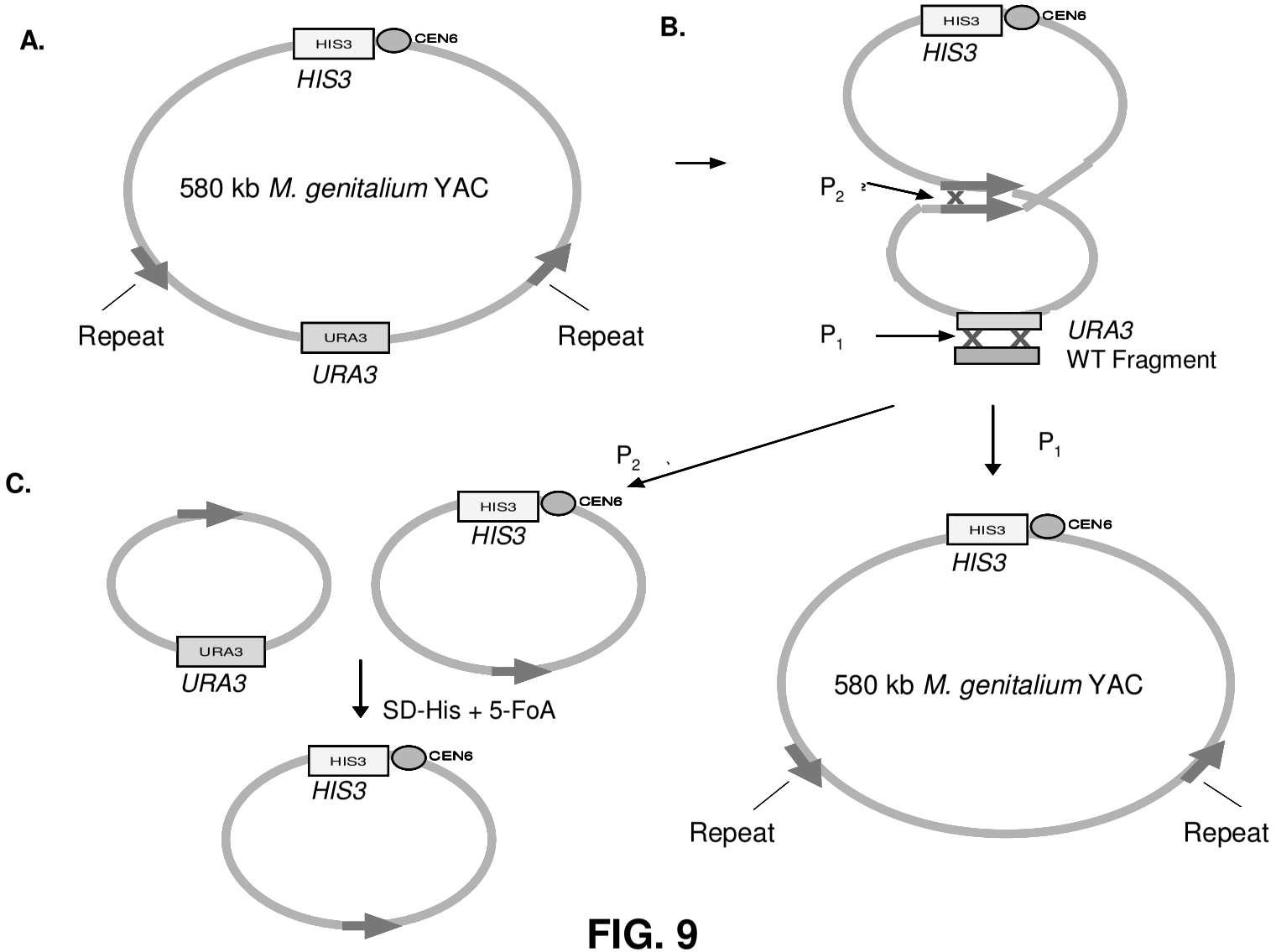

A.
HIS3
CEN6
HIS3
580 kb M. genitalium YAC
Repeat
URA3
URA3
Repeat
B.
HIS3
CEN6
HIS3
P2
P1
URA3
WT Fragment
C.
P2
P1
HIS3
CEN6
HIS3
URA3
URA3
SD-His + 5-FoA
HIS3
CEN6
HIS3
HIS3
CEN6
HIS3
580 kb M. genitalium YAC
Repeat
Repeat

FIG. 9

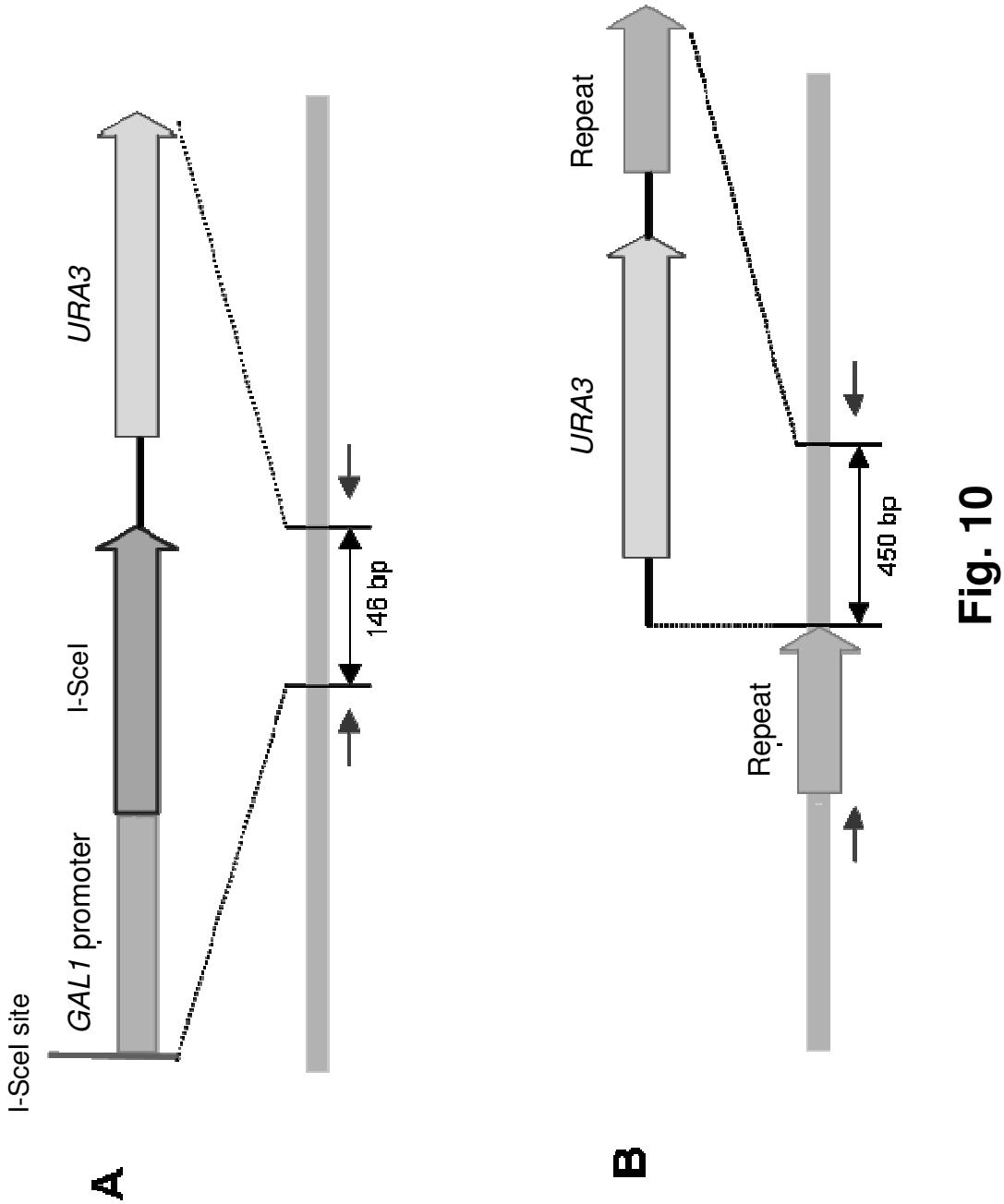

A
I-SceI site
GAL1 promoter
I-SceI
URA3
146 bp
B
Repeat
URA3
Repeat
450 bp

Fig. 10

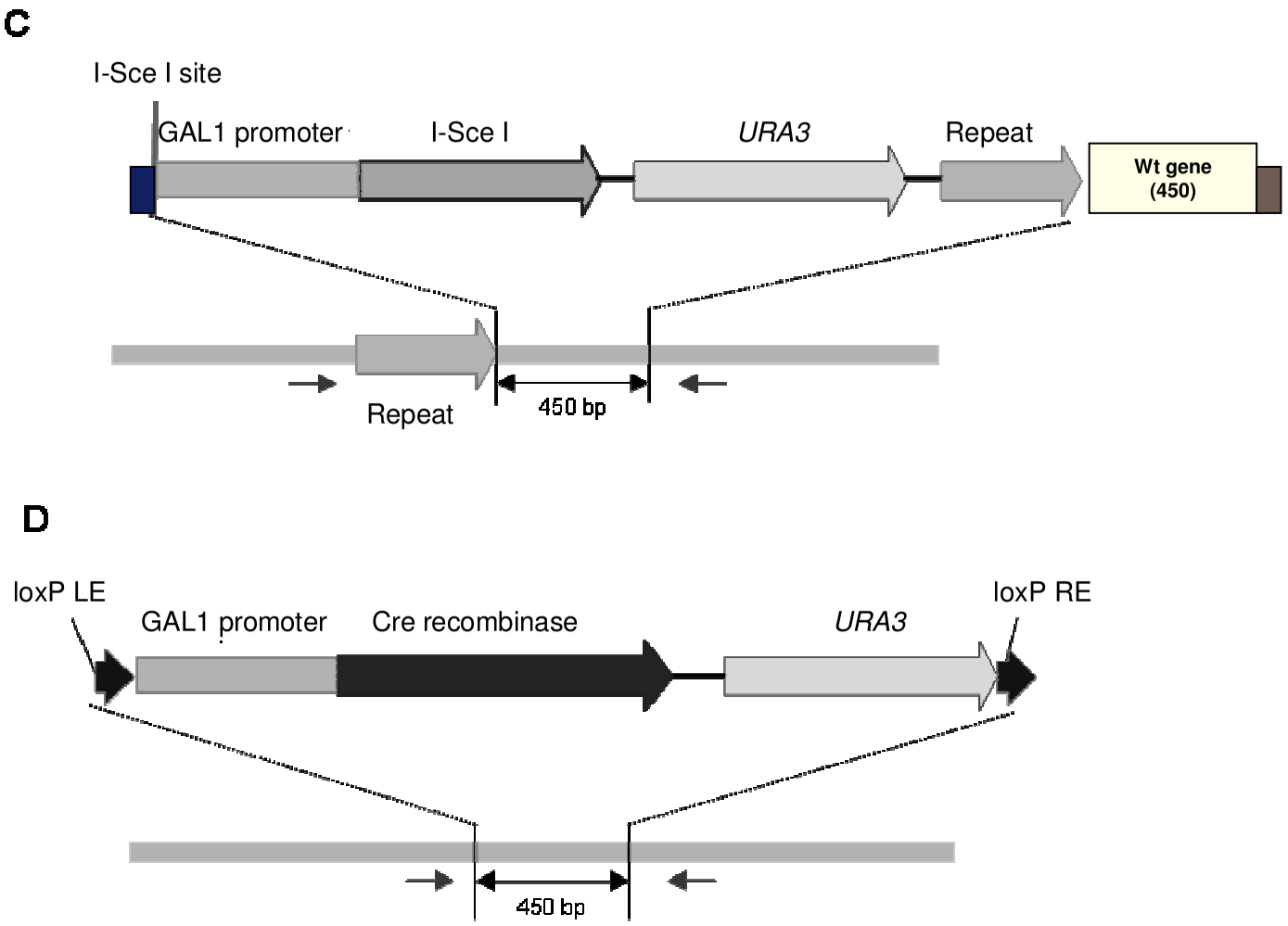

C
I-Sce I site
GAL1 promoter
I-Sce I
URA3
Repeat
Wt gene
(450)
Repeat
450 bp
D
loxP LE
GAL1 promoter
Cre recombinase
URA3
loxP RE
450 bp

FIG. 10

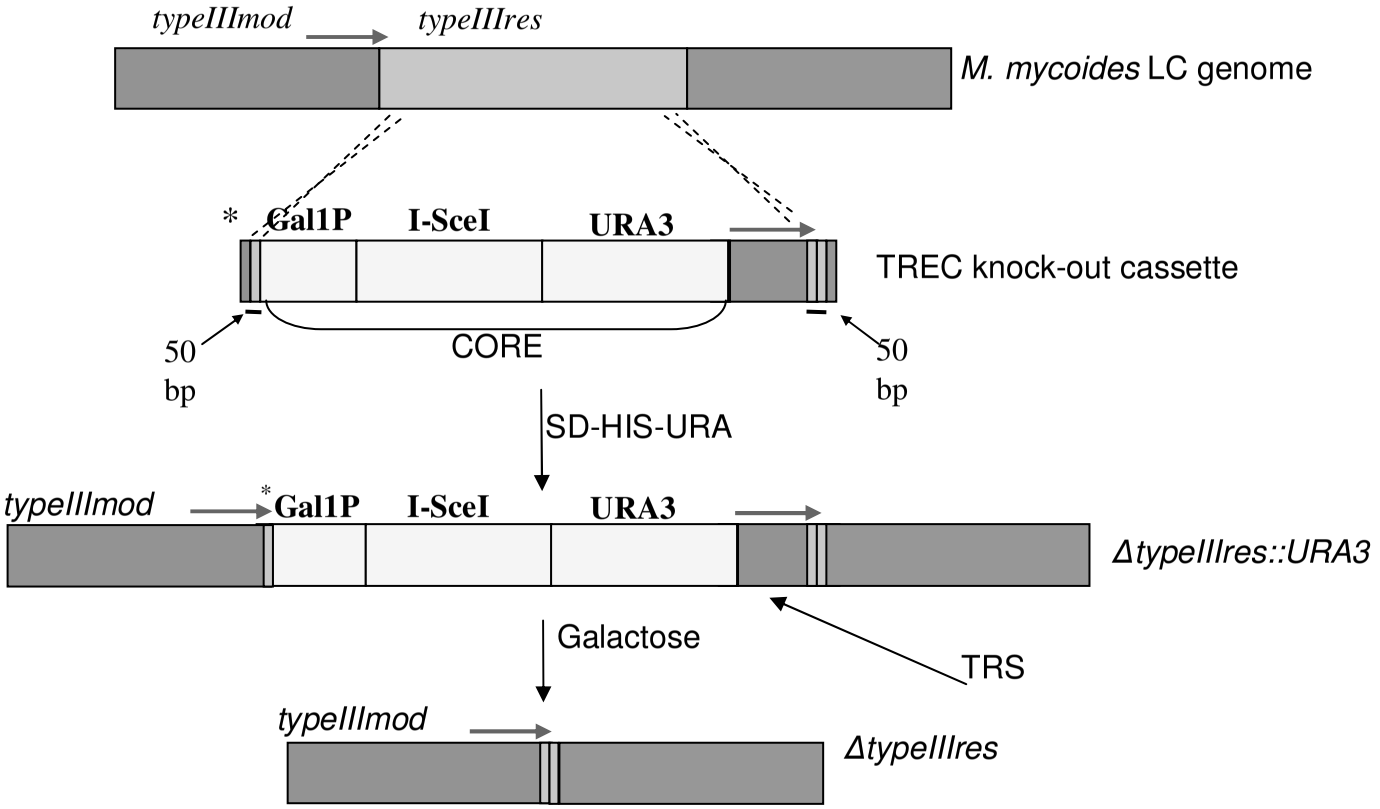

typeIIImod
typeIIIres
M. mycoides LC genome
*
Gal1P
I-SceI
URA3
TREC knock-out cassette
50 bp
CORE
50 bp
SD-HIS-URA
typeIIImod
*
Gal1P
I-SceI
URA3
ΔtypeIIIres::URA3
Galactose
TRS
typeIIImod
ΔtypeIIIres

FIG. 11

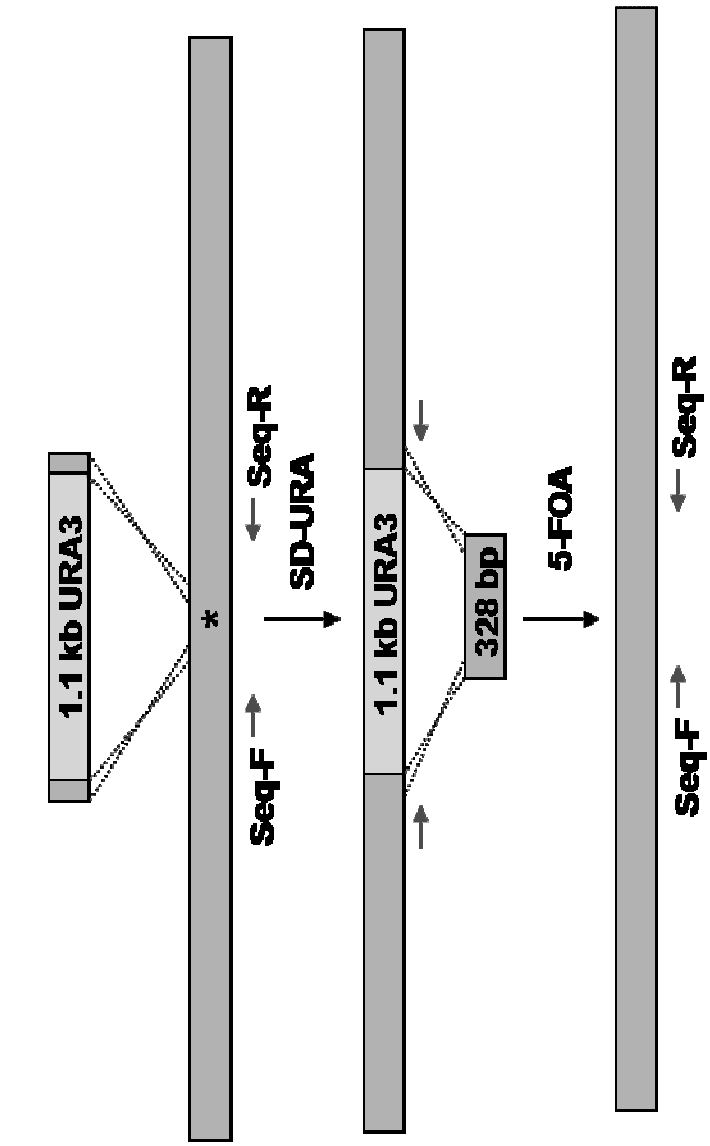


FIG. 12

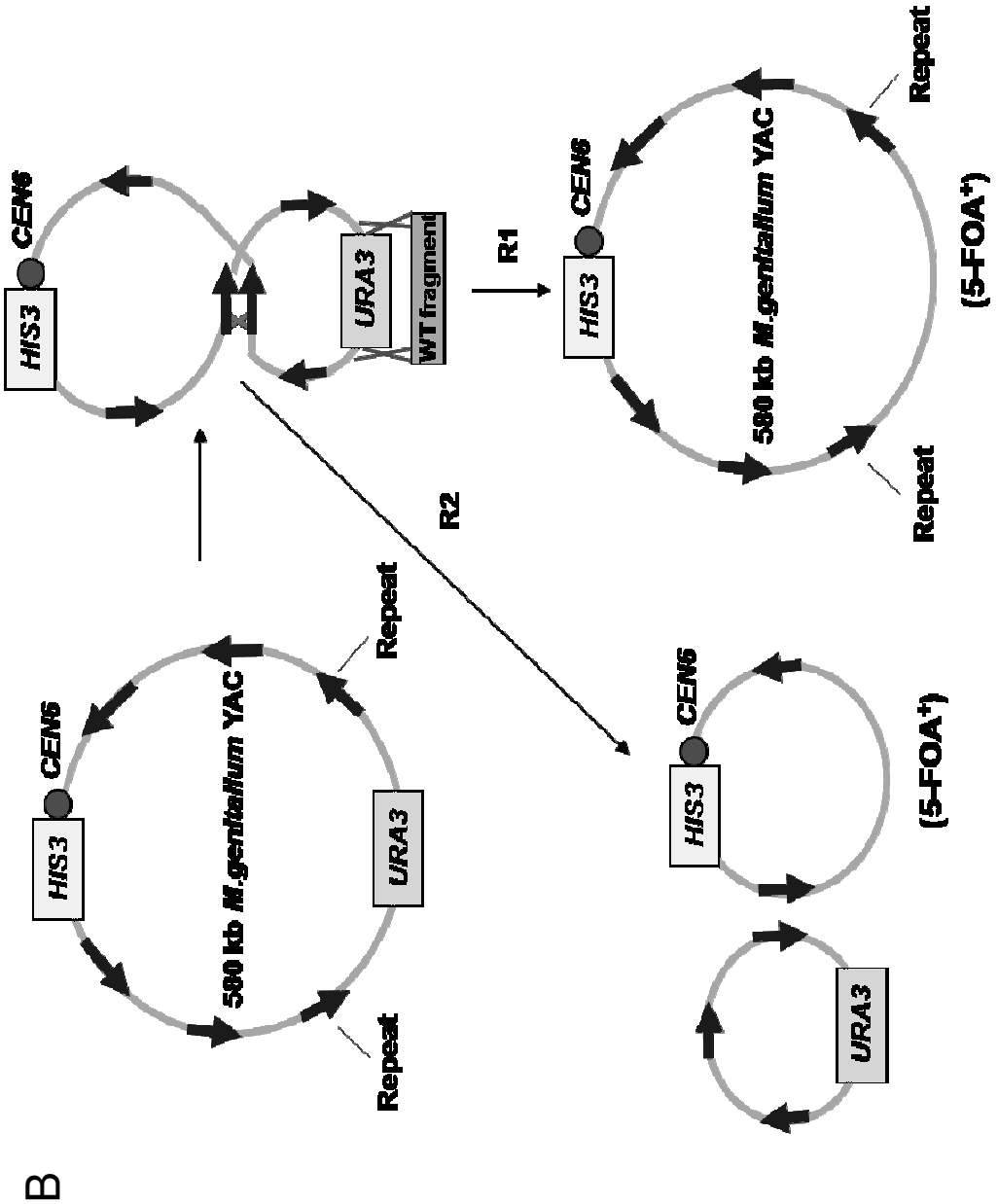

B
CEN6
HIS3
URA3
WT fragment
R1
R2
580 kb M.genitalium YAC
Repeat
(5-FOA+)

FIG. 12

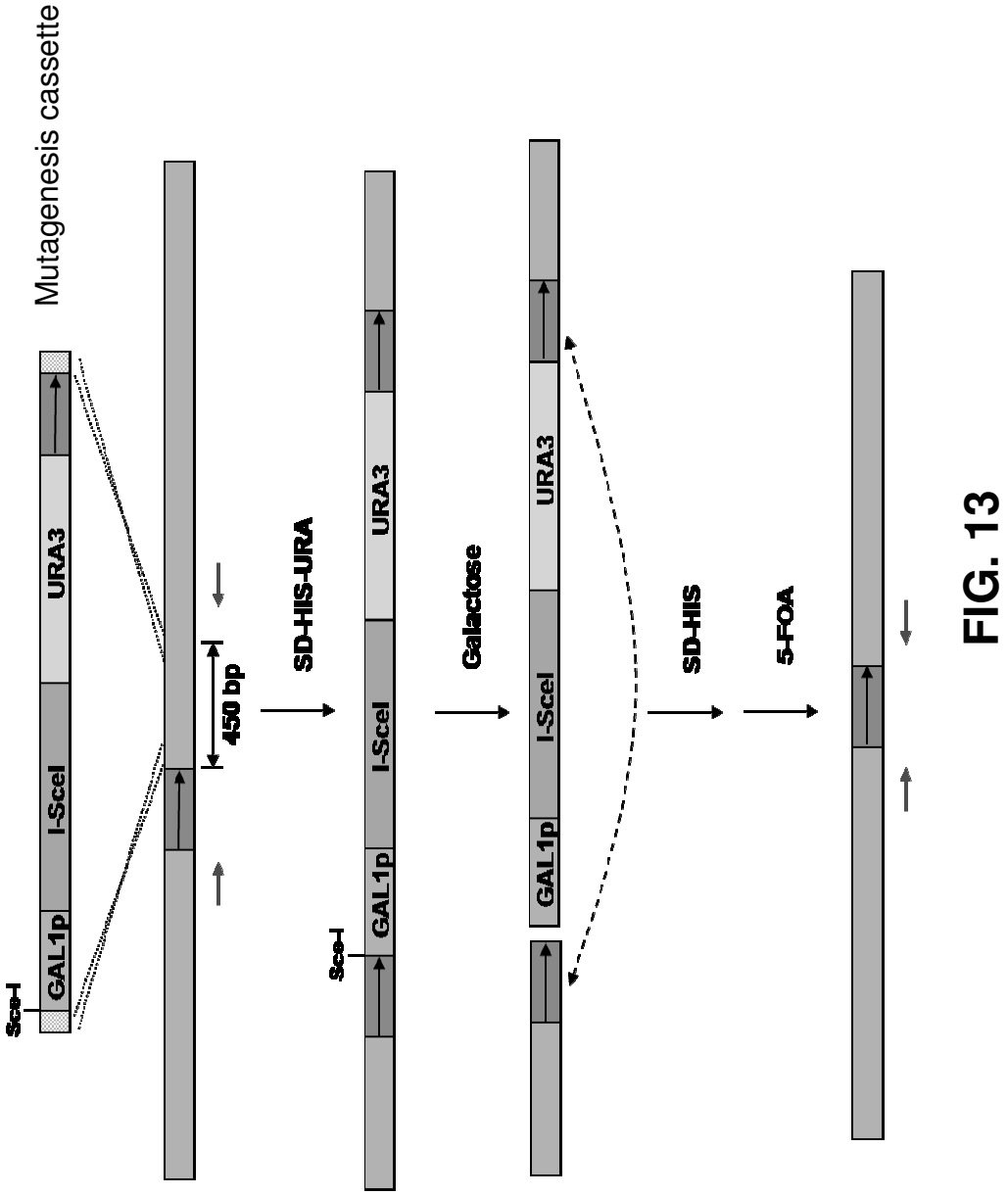

Mutagenesis cassette
SceI
GAL1p
I-SceI
URA3
450 bp
SD-HIS-URA
SceI
GAL1p
I-SceI
URA3
Galactose
GAL1p
I-SceI
URA3
SD-HIS
5-FOA

FIG. 13

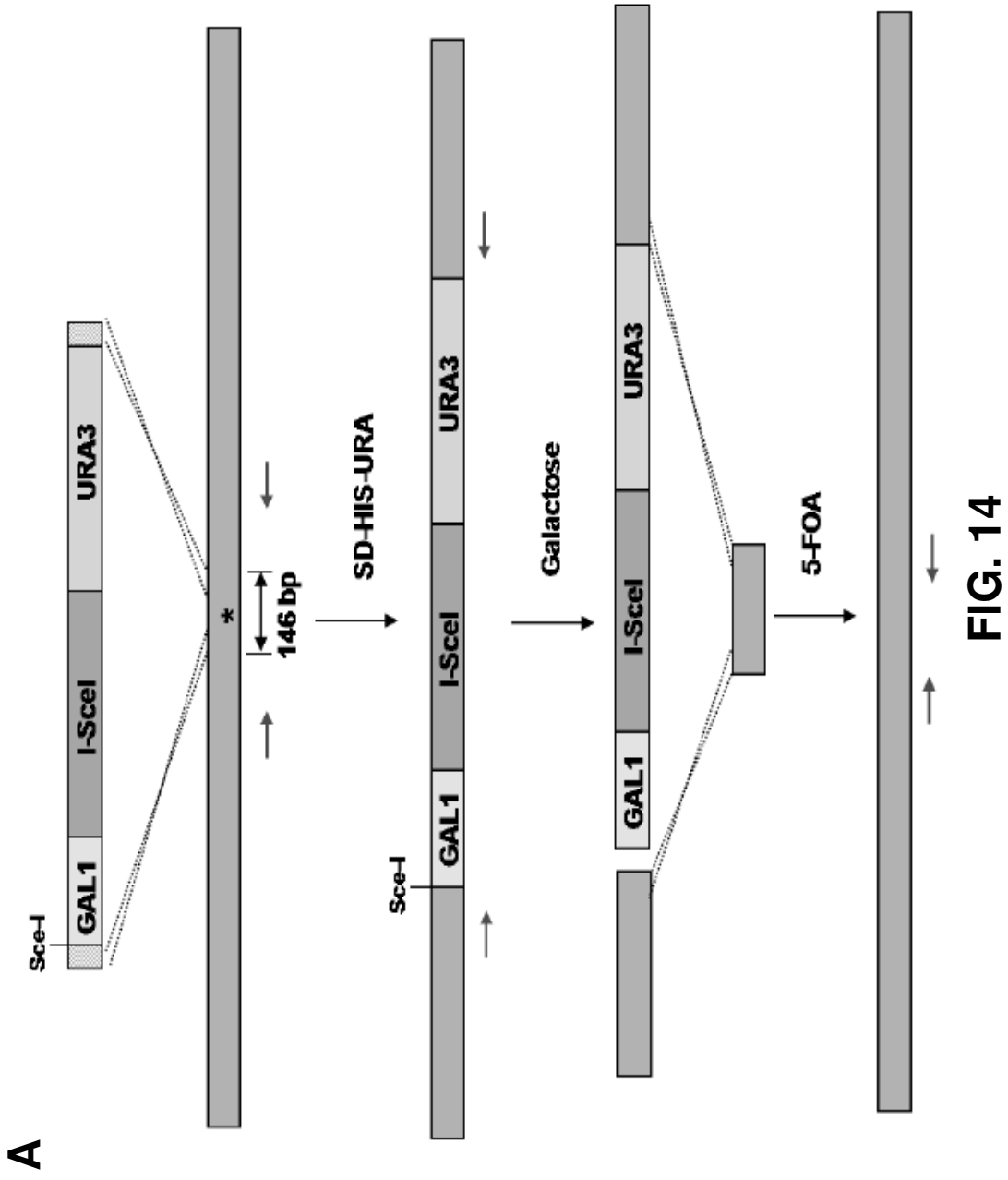


FIG. 14

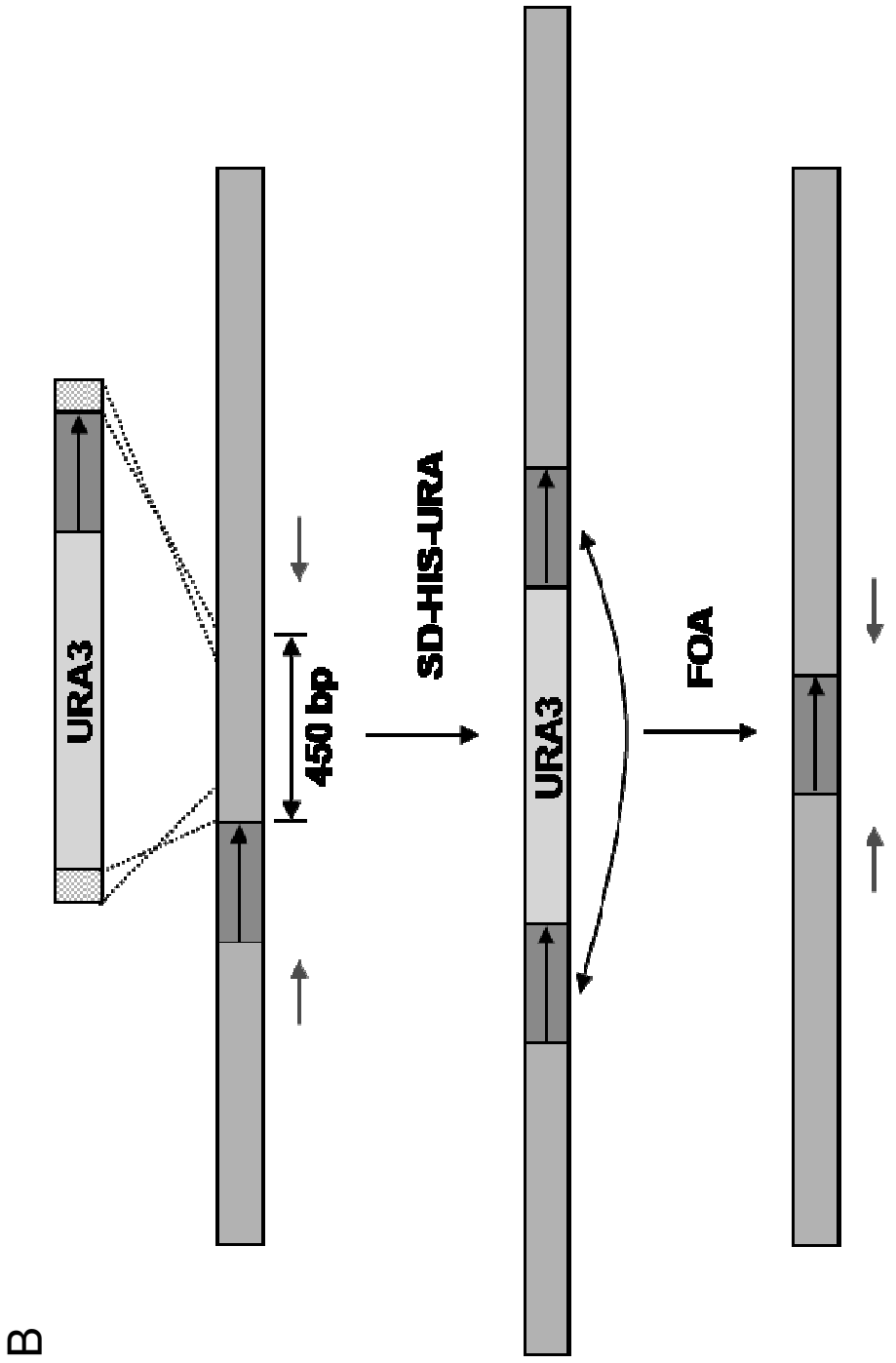


FIG. 14

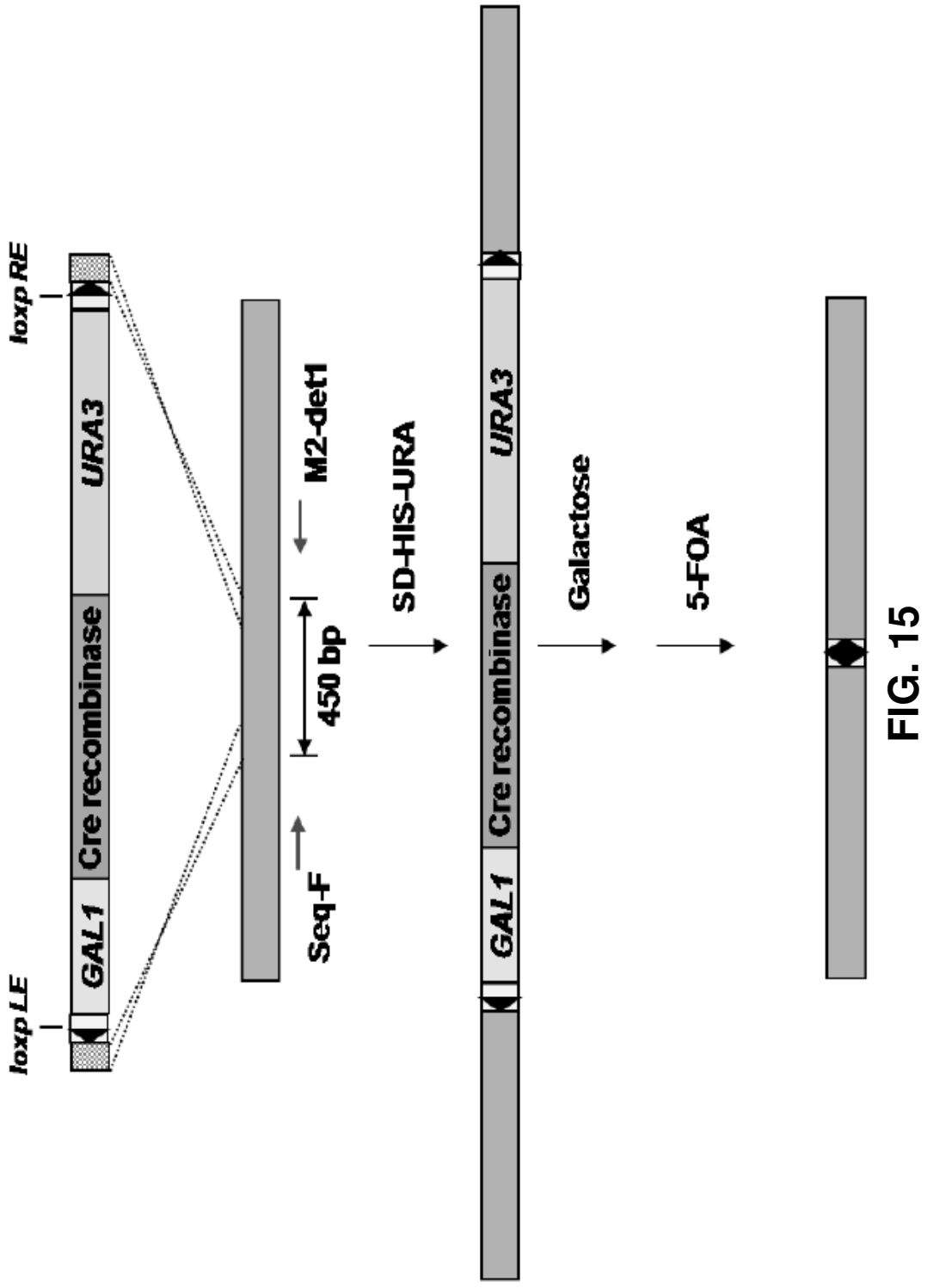

loxp RE
loxp LE
GAL1
Cre recombinase
URA3
M2-del1
450 bp
Seq-F
SD-HIS-URA
GAL1
Cre recombinase
URA3
Galactose
5-FOA

FIG. 15

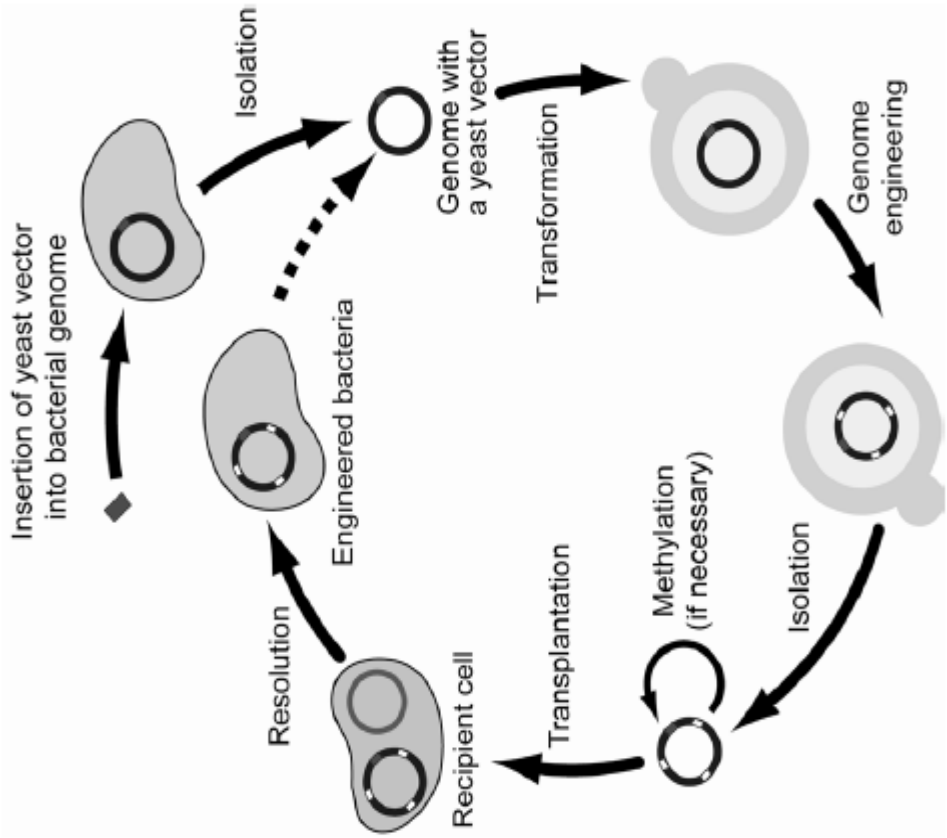

Insertion of yeast vector into bacterial genome
Isolation
Genome with a yeast vector
Transformation
Genome engineering
Isolation
Methylation (if necessary)
Transplantation
Recipient cell
Resolution
Engineered bacteria

FIG. 16

AGGAAGGCTGTGTGAATTTCATTCGACAAGATAAAGGAAATTAGAACATTTACTTTAGTTACAAATAATGAAAA

TAATATAGGGATTGATGTTTGCTATGAACGCTTGTTTAGAATAAATAATGGTAAATCAACTGATAATAAAACAG

ATTTTAAATGAATAGAAAAAAACGAACCCTATCTGTCTAATTTAGATGTTTTTGATATTAAATACTATTCAACT

AAATTGTTTGAAGATAATAGTGCTAATGAAATAATCAAAAAACAATTCATAAAAATGTTAAATGATCTAAAAAT

AAATGCTGATGATATTTCTACAATTAAAACTTTAAGACAATTAACTGCACTAAAACCAATCTCAGGTGGACAAA

*ACA*ATGAGATTAAC***TAA***TAAACAAGAATTTGTAGTGCAAGATATTGTTAAAGAAGATTATTAA**ATTGTTA**

AAATAAAAAATTACTTATTTAAGCGGGACTATTTCTATTTTATTTAAAGAAGTCTTATGATTGAATGTAAAAGT

TCTAATGTATTAACCCCTGTTACATTAAATACAACCATATCTATTTTTAATTAATCTCCTGAAAGTACAAGTTC

TTAATTCCTAACCATGCCTATTAAATTTAAGATCAAGAGCAGTTTTTCTAACCCCCGAAAAAAAACTCACAACA

AAAA

(SEQ ID NO: 195)

FIG. 17

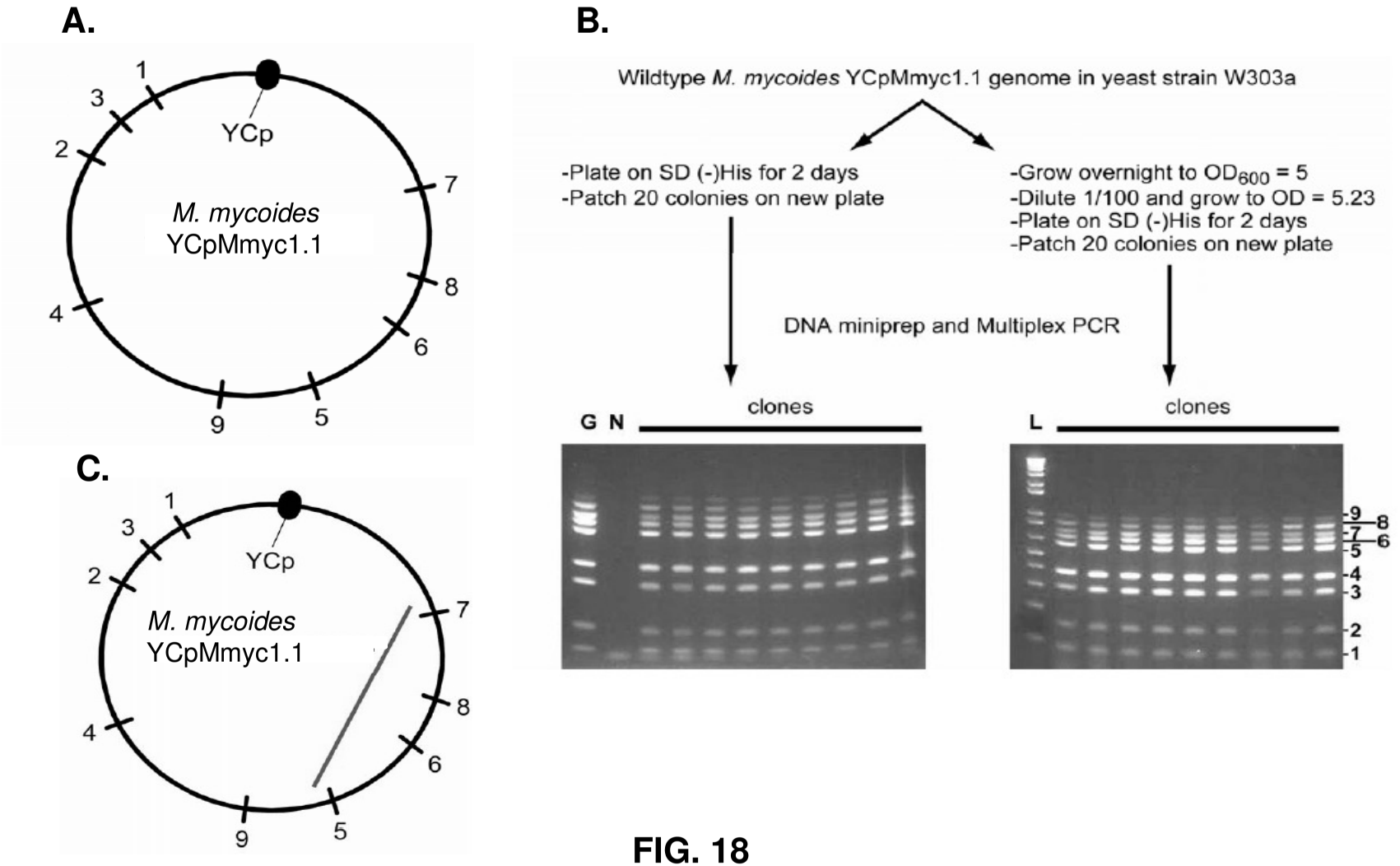

A.
1
3
2
YCp
7
M. mycoides
YCpMmyc1.1
8
4
6
9
5
B.
Wildtype M. mycoides YCpMmyc1.1 genome in yeast strain W303a
-Plate on SD (-)His for 2 days
-Patch 20 colonies on new plate
-Grow overnight to OD600 = 5
-Dilute 1/100 and grow to OD = 5.23
-Plate on SD (-)His for 2 days
-Patch 20 colonies on new plate
DNA miniprep and Multiplex PCR
clones
G N
clones
L
9
8
7
6
5
4
3
2
1
C.
1
3
2
YCp
7
M. mycoides
YCpMmyc1.1
8
4
6
9
5

FIG. 18

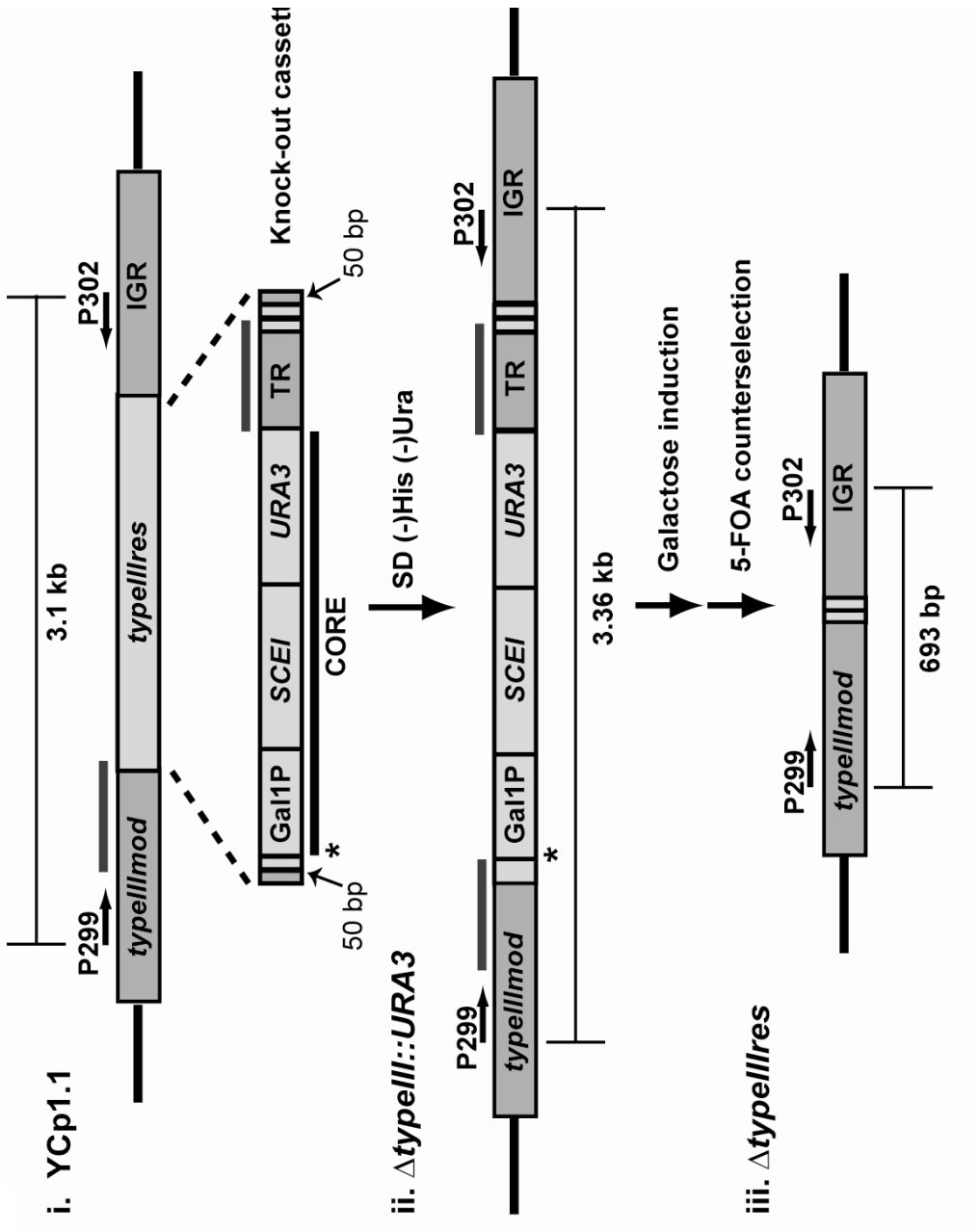

i. YCp1.1
3.1 kb
P299
typeIIImod
typeIIIres
P302
IGR
Knock-out cassett
50 bp
TR
URA3
SCEI
Gal1P
CORE
*
50 bp
ii. ΔtypeIII::URA3
SD (-)His (-)Ura
P299
typeIIImod
Gal1P
SCEI
URA3
TR
P302
IGR
*
3.36 kb
Galactose induction
5-FOA counterselection
iii. ΔtypeIIIres
P299
typeIIImod
P302
IGR
693 bp

FIG. 19