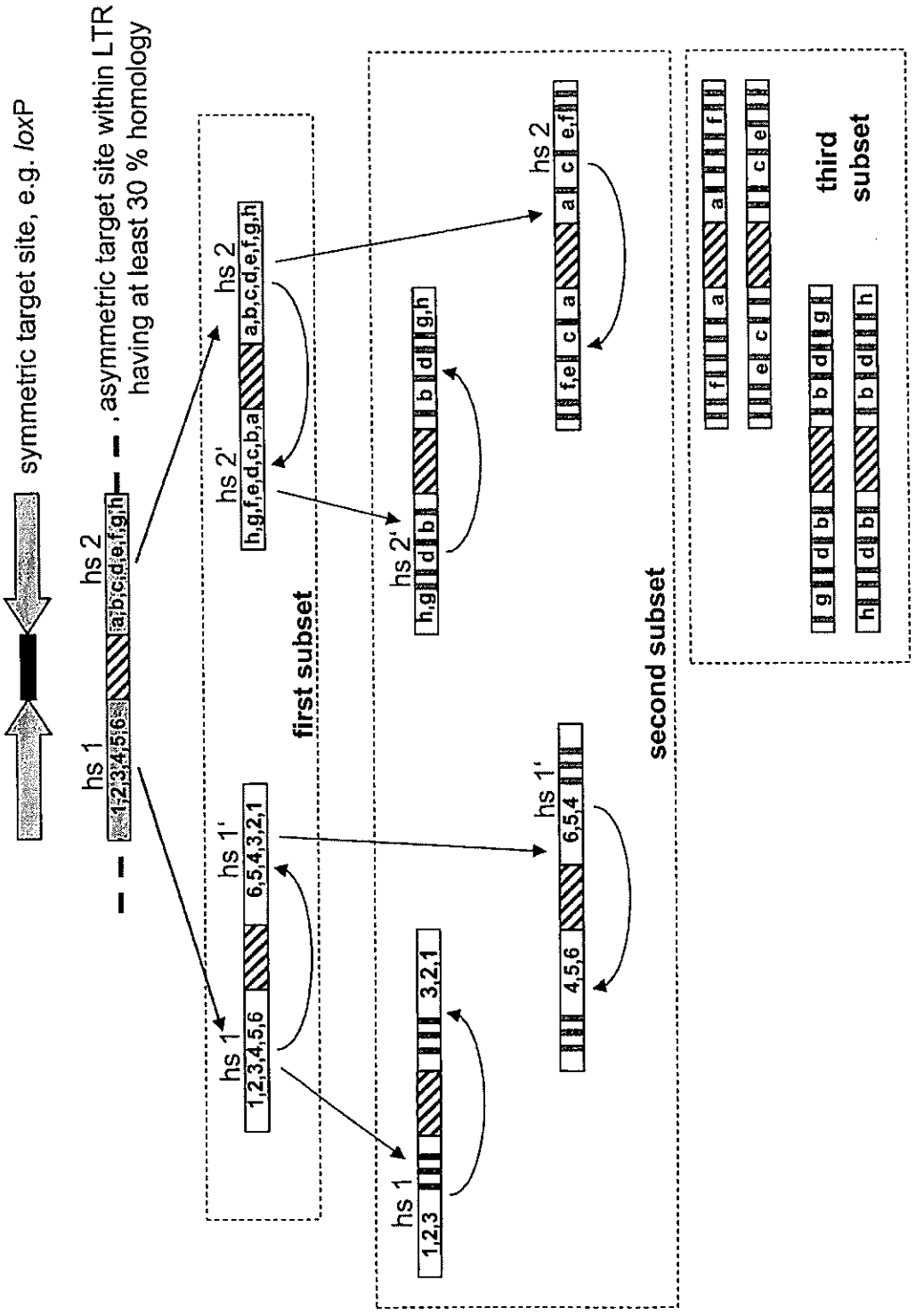

symmetric target site, e.g. loxP
asymmetric target site within LTR
having at least 30 % homology
hs 1
1,2,3,4,5,6
hs 2
a,b,c,d,e,f,g,h
hs 1'
6,5,4,3,2,1
hs 2'
h,g,f,e,d,c,b,a
first subset
second subset
third subset
1,2,3,4,5,6
a,b,c,d,e,f,g,h
h,g,f,e,d,c,b,a
6,5,4,3,2,1
1,2,3
3,2,1
4,5,6
h,g
g,h
f,e
e,f

Figure 1

A

ATAACTTCGTATA ATGTATGC TATACGAAGTTAT	LoxP
ACAACATCCTATT ACACCCTA TATGCCAACATGG	LoxLTR
ACAACATCCTATT ACACCCTA AATAGGATGTTGT	LoxLTR1
ACAACATCGTATA ACACCCTA TATACGATGTTGT	LoxLTR1a
ATAACTTCCTATT ACACCCTA AATAGGAAGTTAT	LoxLTR1b
CCATGTTGGCATA ACACCCTA TATGCCAACATGG	LoxLTR2
CCATCTTCGTATA ACACCCTA TATACGAAGATGG	LoxLTR2a
ATAAGTTGGCATA ACACCCTA TATGCCAACTTAT	LoxLTR2b

B

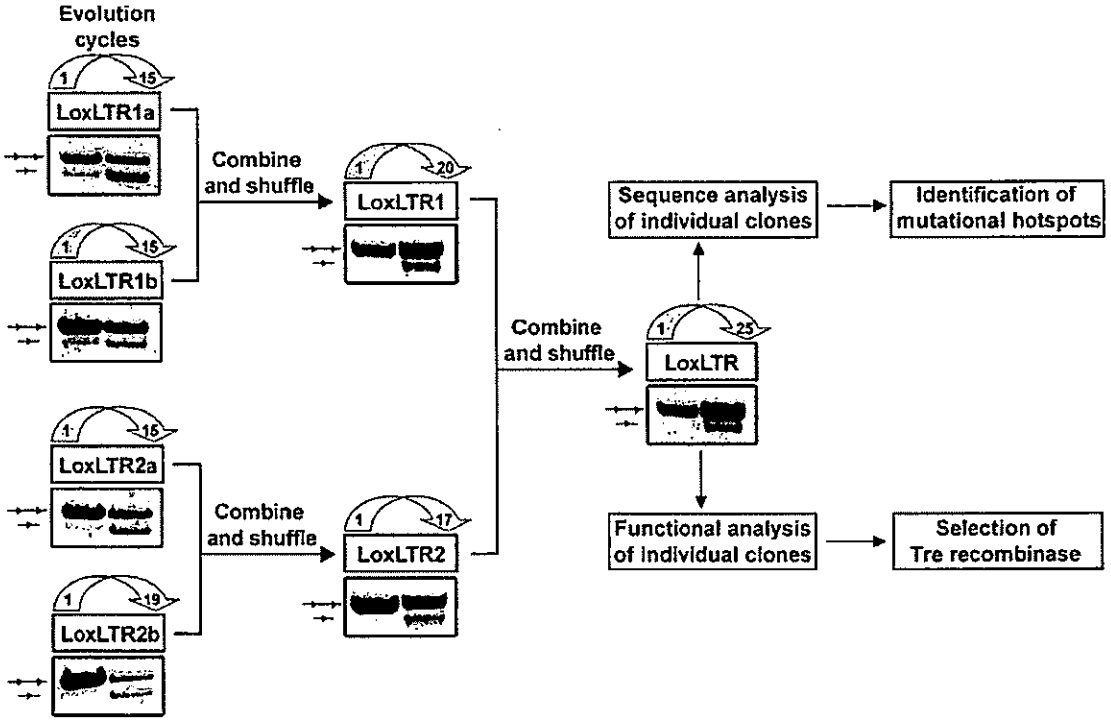


Figure 2

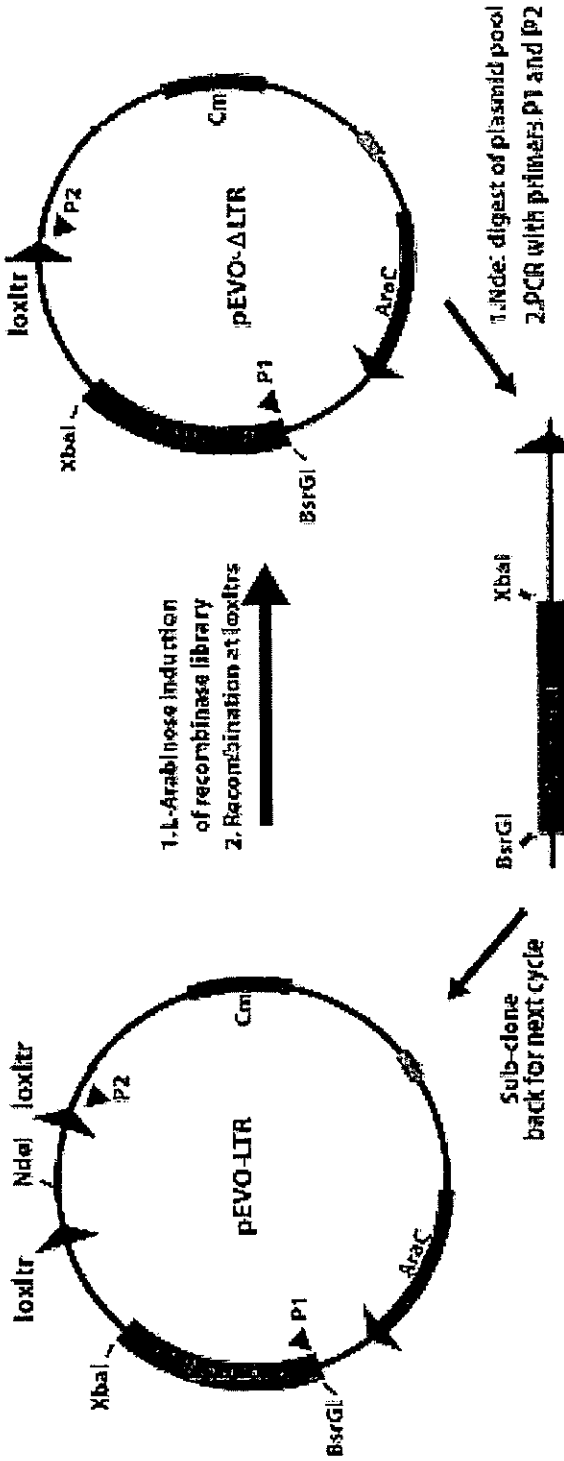

pEVO-ΔLTR
loxltr
P2
Cm
AraC
P1
XbaI
BsrGI
1.NdeI digest of plasmid pool
2.PCR with primers P1 and P2
XbaI
BsrGI
PCR product
(1.7 kb)
1.L-Arabinose induction
of recombinase library
2. Recombination at loxltrs
Sub-clone
back for next cycle
pEVO-LTR
loxltr
NdeI
P2
Cm
AraC
P1
XbaI
BsrGI

Figure 3

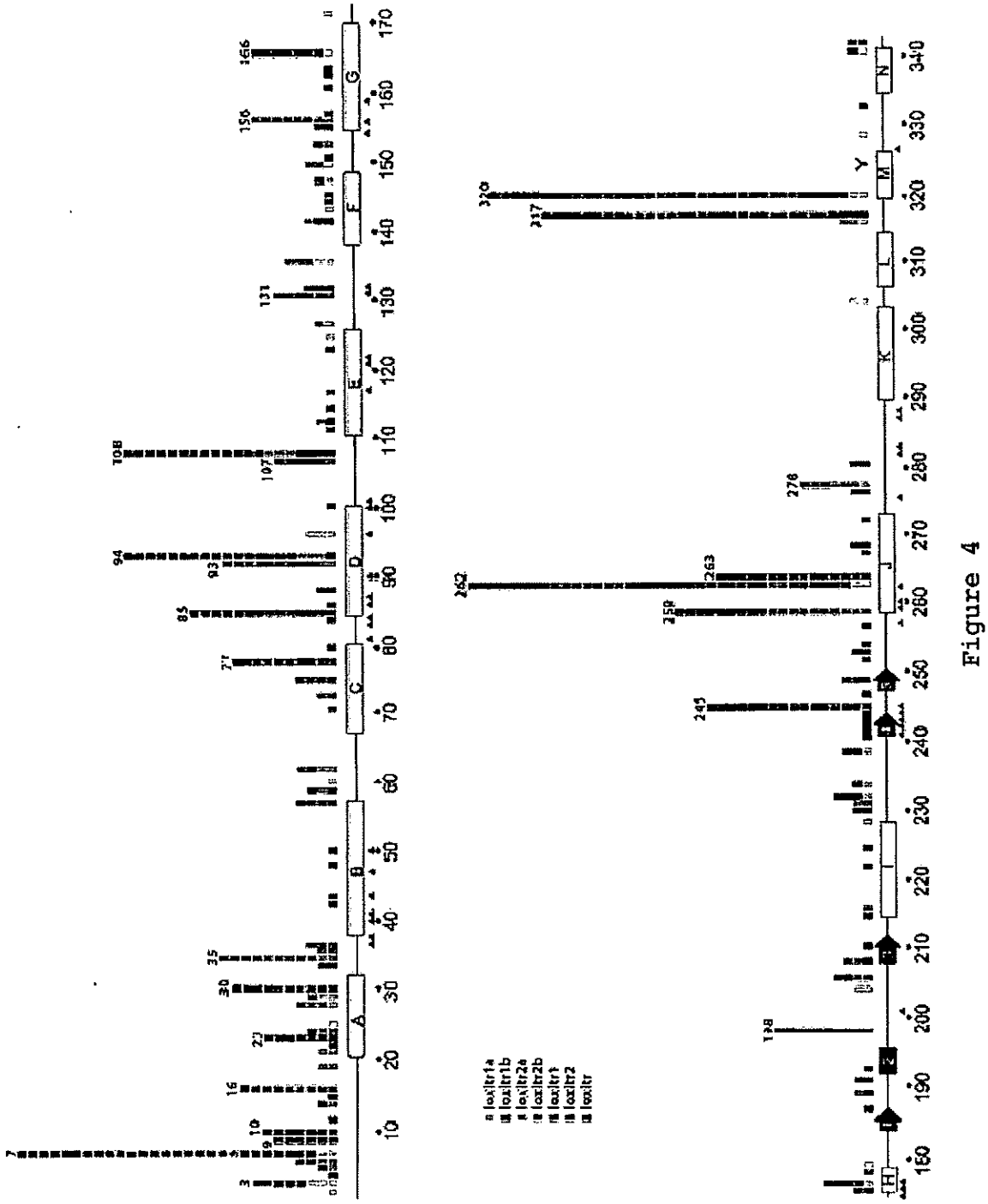

loxitr1a
loxitr1b
loxitr2a
loxitr2b
loxitr1
loxitr2
loxitr

Figure 4

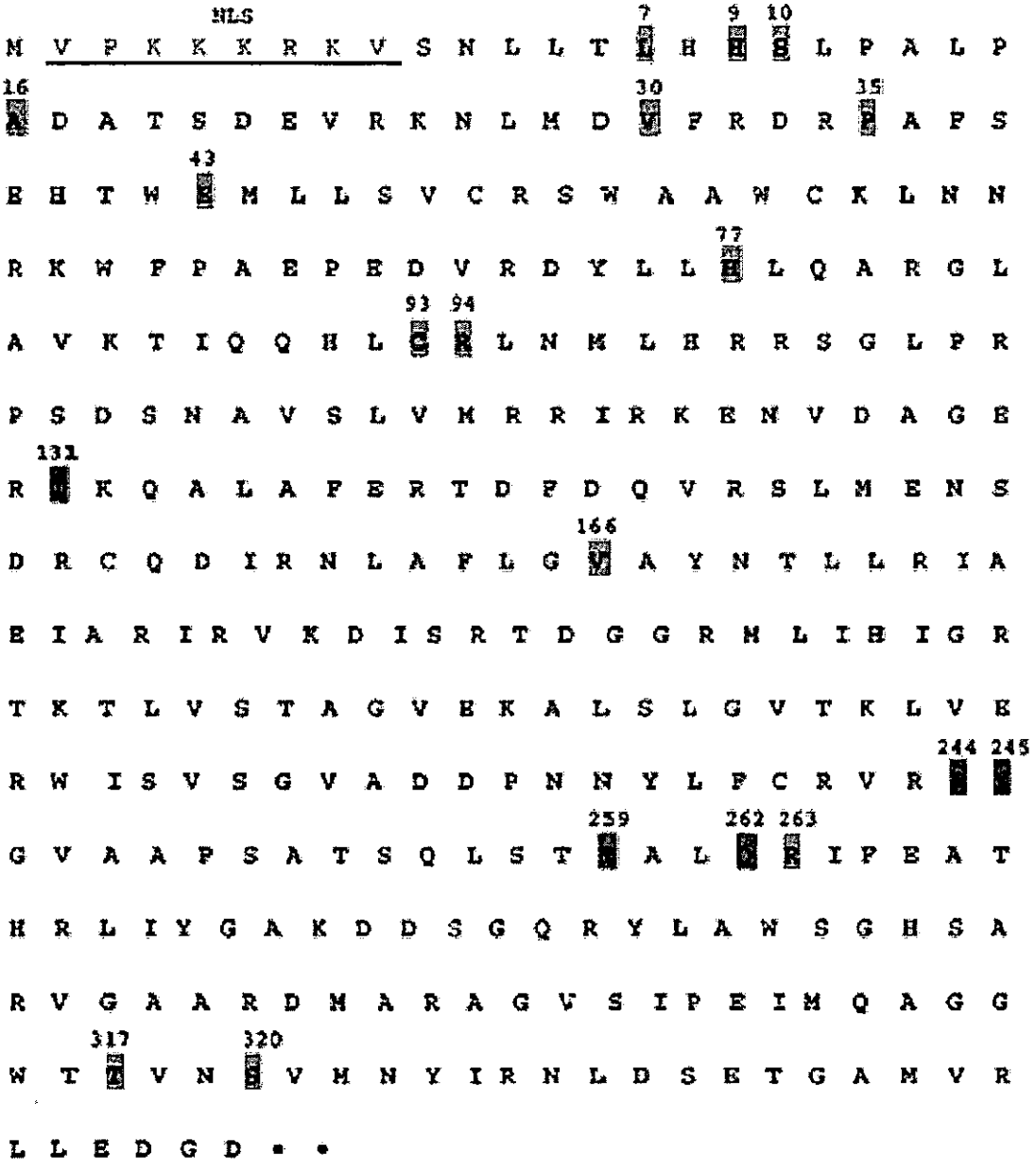


Figure 5

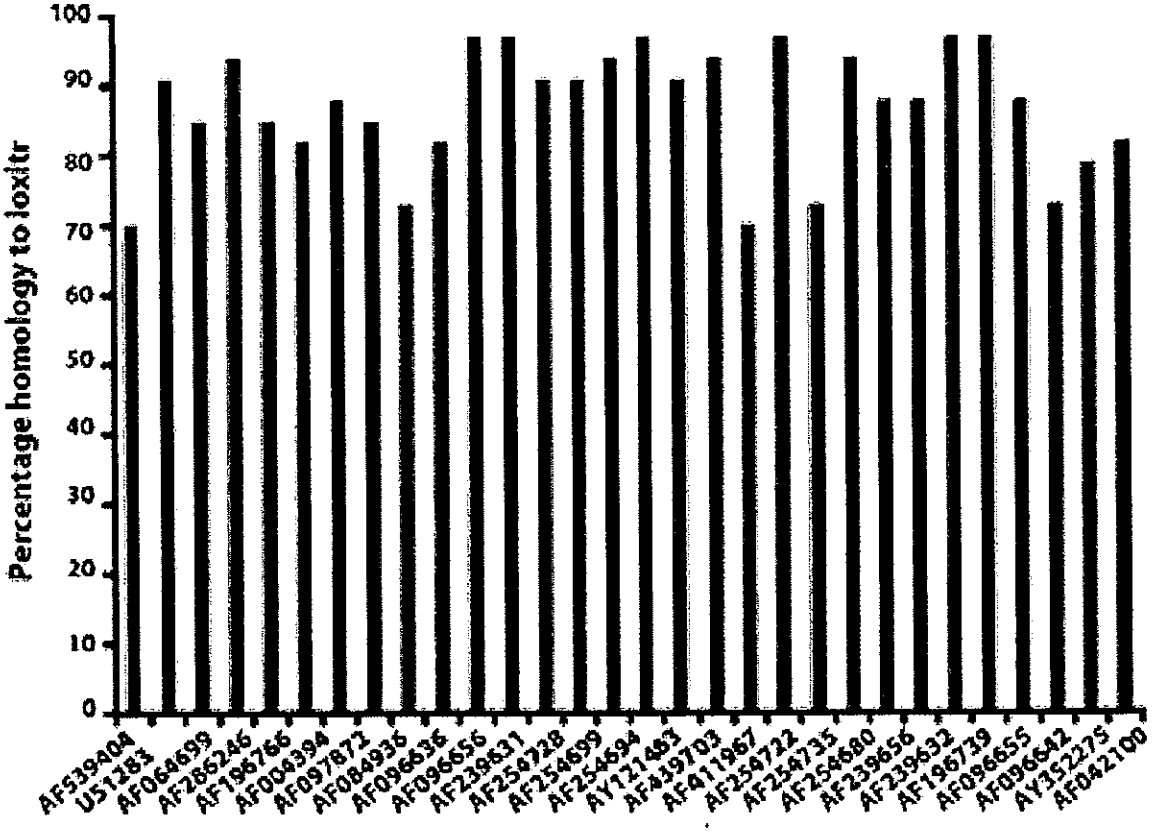

Percentage homology to ioxitr
100
90
80
70
60
50
40
30
20
10
0
AF539404
U51283
AF064699
AF286246
AF196766
AF004394
AF097872
AF084936
AF096636
AF096656
AF239631
AF254728
AF254699
AF254694
AY121463
AF419703
AF411967
AF254732
AF254735
AF254680
AF239656
AF239632
AF196739
AF096655
AF096642
AY352275
AF042100

Figure 6

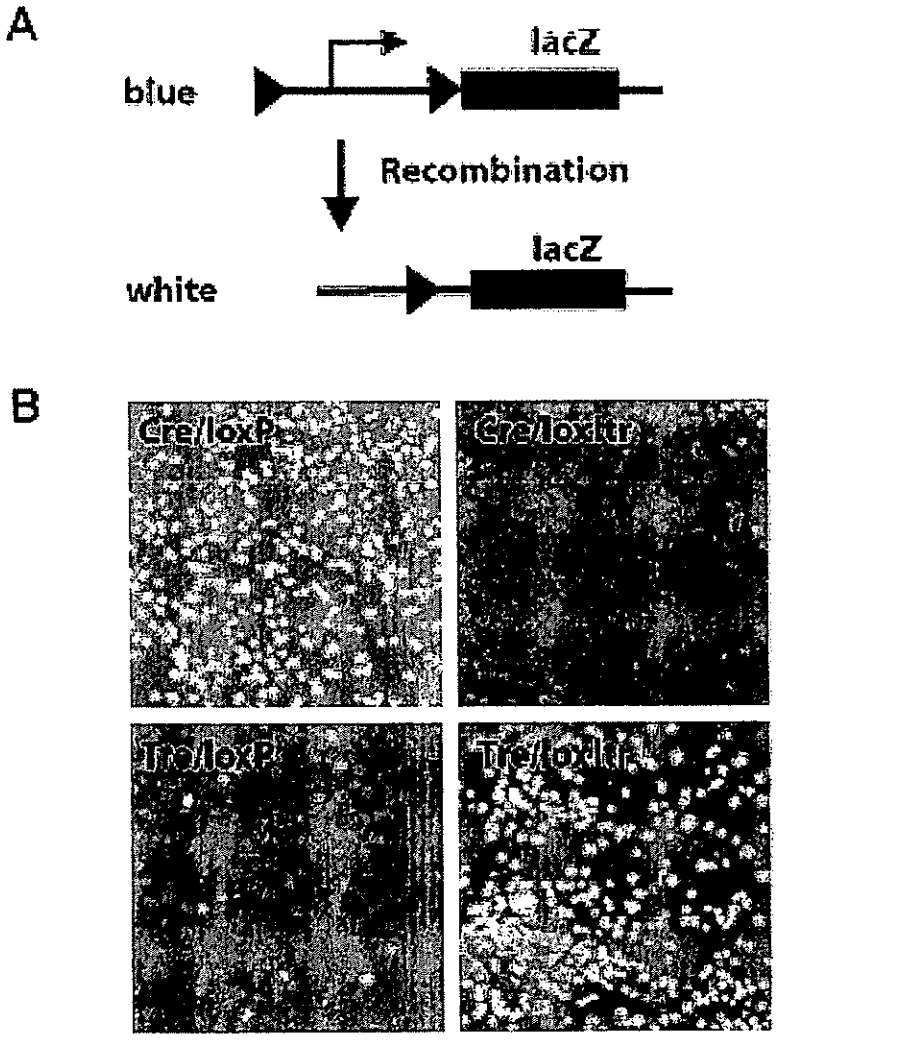

A
lacZ
blue
Recombination
lacZ
white
B
Cre/loxP
Cre/loxtr
Tre/loxP
Tre/loxtr
C
L
loxtr
loxP
loxtr1a
loxtr1b
loxtr1
loxtr2a
loxtr2b
loxtrZ
4.9kb
4.2kb
% 94 8 0 0 0 0 72 72

Figure 7

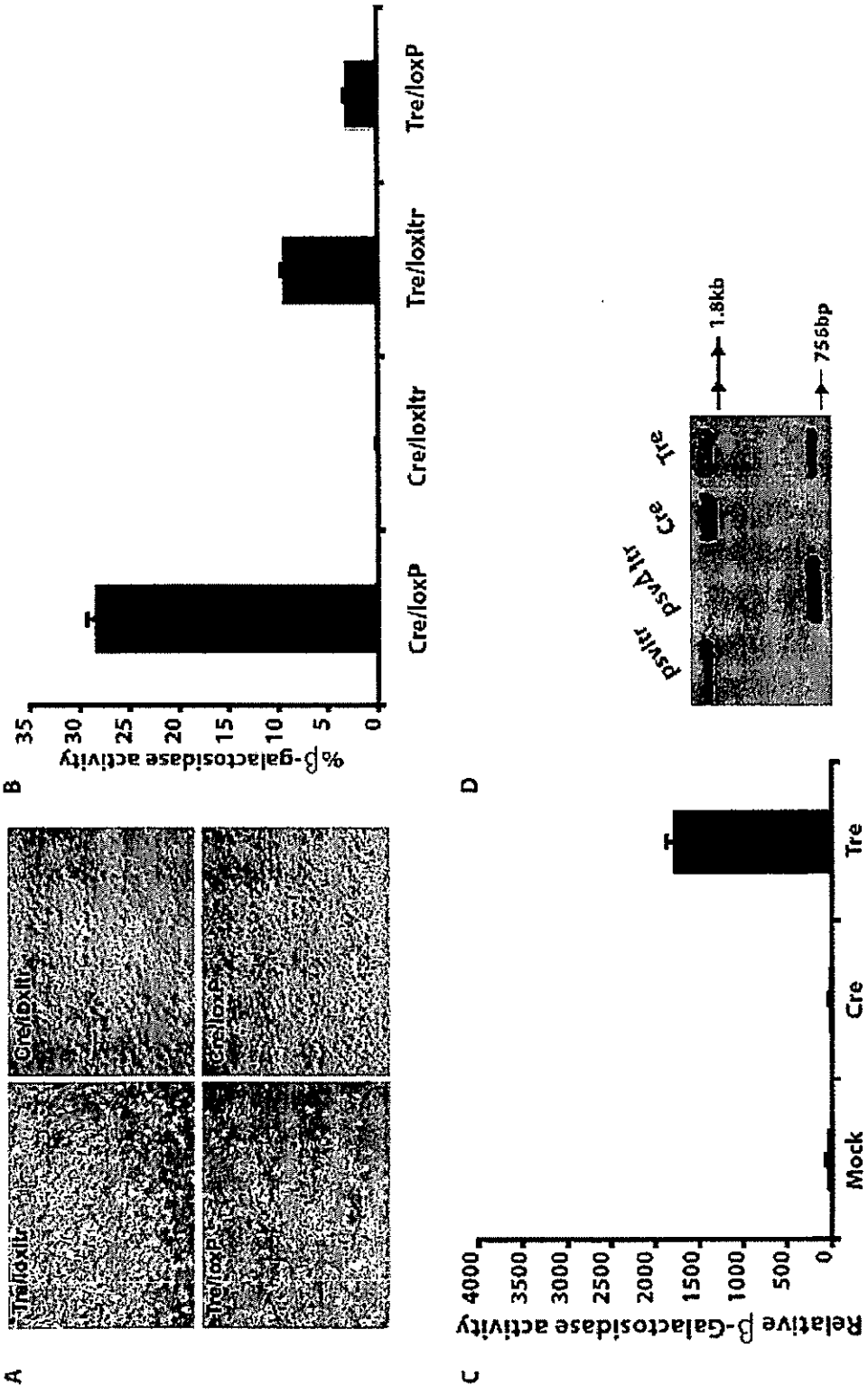

A
Tre/loxitr
Cre/loxitr
Tre/loxP
Cre/loxP
B
%β-galactosidase activity
0
5
10
15
20
25
30
35
Cre/loxP
Cre/loxitr
Tre/loxitr
Tre/loxP
C
Relative β-Galactosidase activity
0
500
1000
1500
2000
2500
3000
3500
4000
Mock
Cre
Tre
D
pSVAtr
pSVAtr
Cre
Tre
1.8kb
756bp

Figure 8

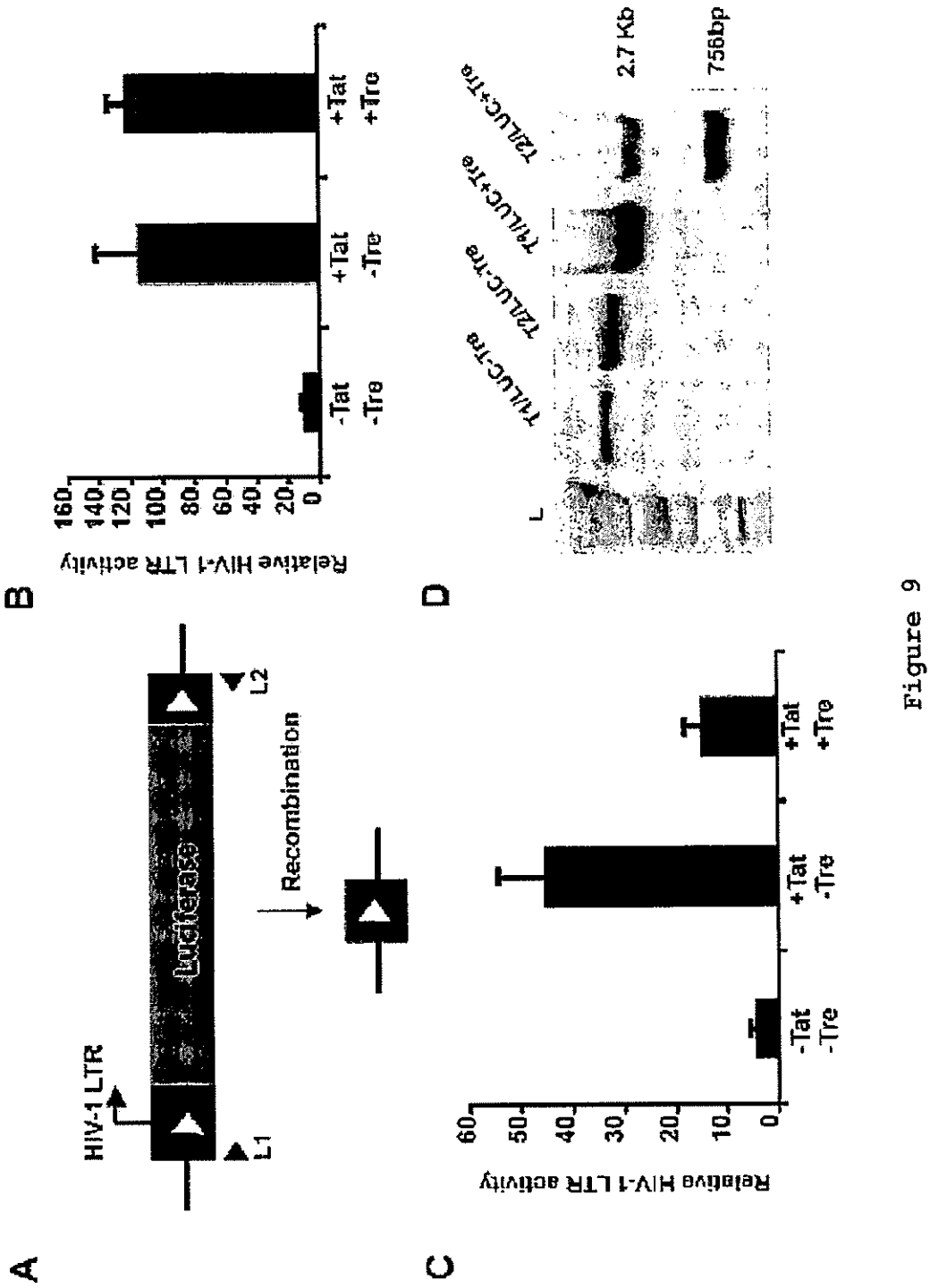

A
HIV-1 LTR
Luciferase
L1
L2
Recombination
B
Relative HIV-1 LTR activity
160
140
120
100
80
60
40
20
0
-Tat -Tre
+Tat -Tre
+Tat +Tre
C
Relative HIV-1 LTR activity
60
50
40
30
20
10
0
-Tat -Tre
+Tat -Tre
+Tat +Tre
D
L
T1iLUC-Tre
T2iLUC-Tre
T4iLUC+Tre
T2iLUC+Tre
2.7 Kb
756bp

Figure 9

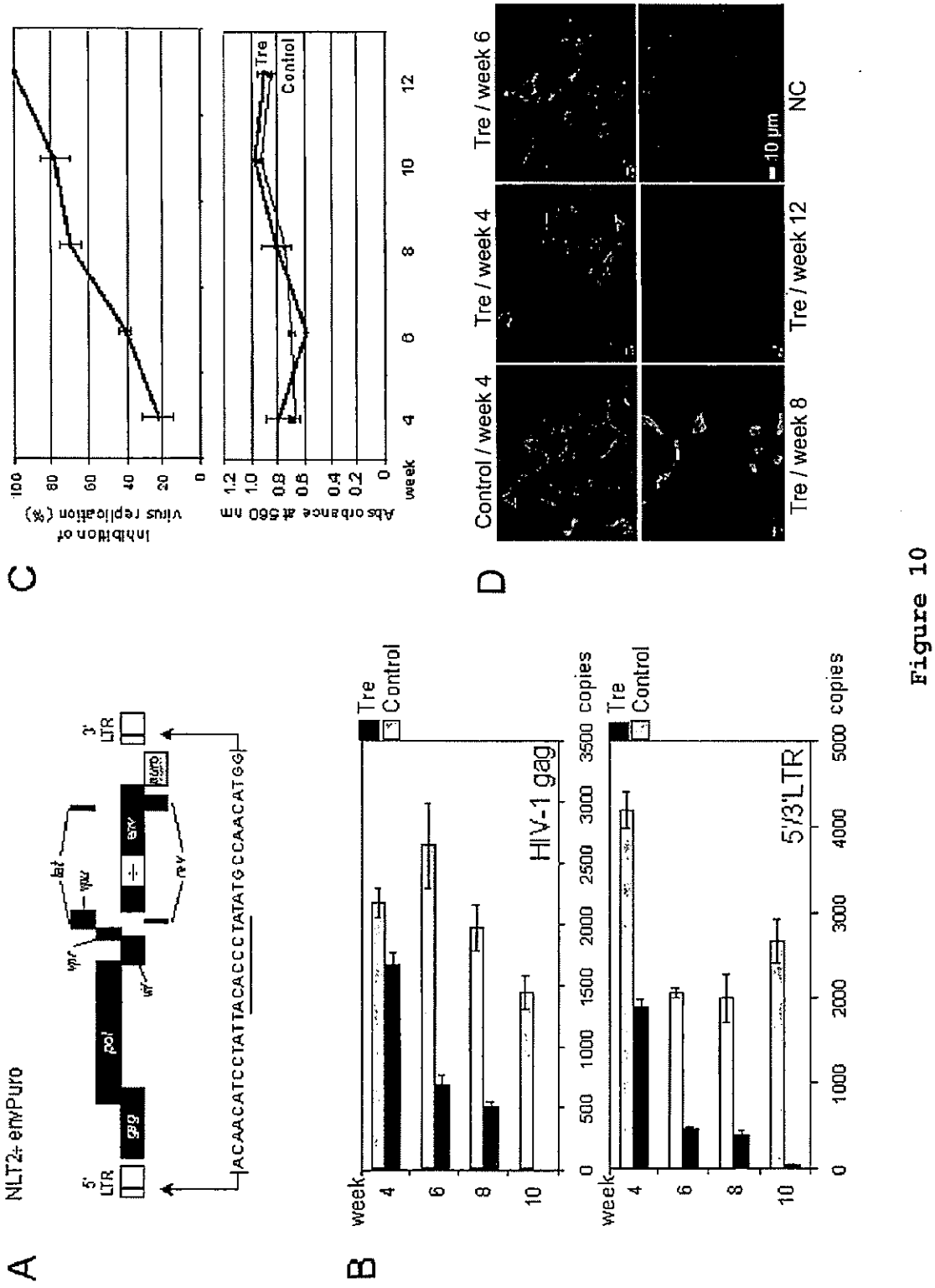

A
NLT2÷ emPuro
5' LTR
gag
pol
vif
vpr
tat
rev
vpu
env
nef
3' LTR
ACAACATCCTATTACACCCTATATGCCAACATGG
B
Tre
Control
week
4
6
8
10
HIV-1 gag
0 500 1000 1500 2000 2500 3000 3500 copies
5'/3'LTR
0 1000 2000 3000 4000 5000 copies
C
Inhibition of virus replication (%)
100 80 60 40 20 0
Absorbance at 560 nm
1.2 1.0 0.8 0.6 0.4 0.2 0
week
0 4 6 8 10 12
D
Control / week 4
Tre / week 4
Tre / week 6
Tre / week 8
Tre / week 12
10 µm
NC

Figure 10

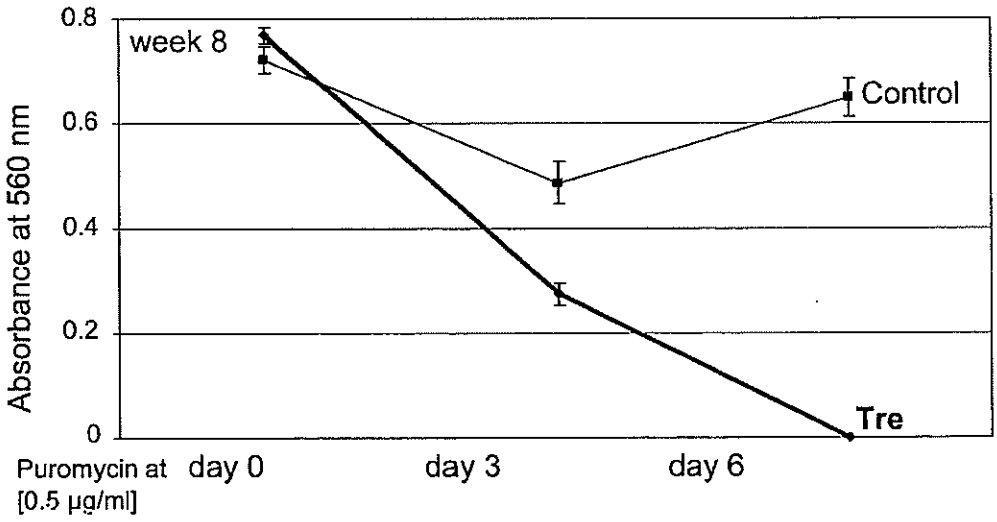

0.8
0.6
0.4
0.2
0
Absorbance at 560 nm
week 8
Control
Tre
Puromycin at
[0.5 µg/ml]
day 0
day 3
day 6

Figure 11