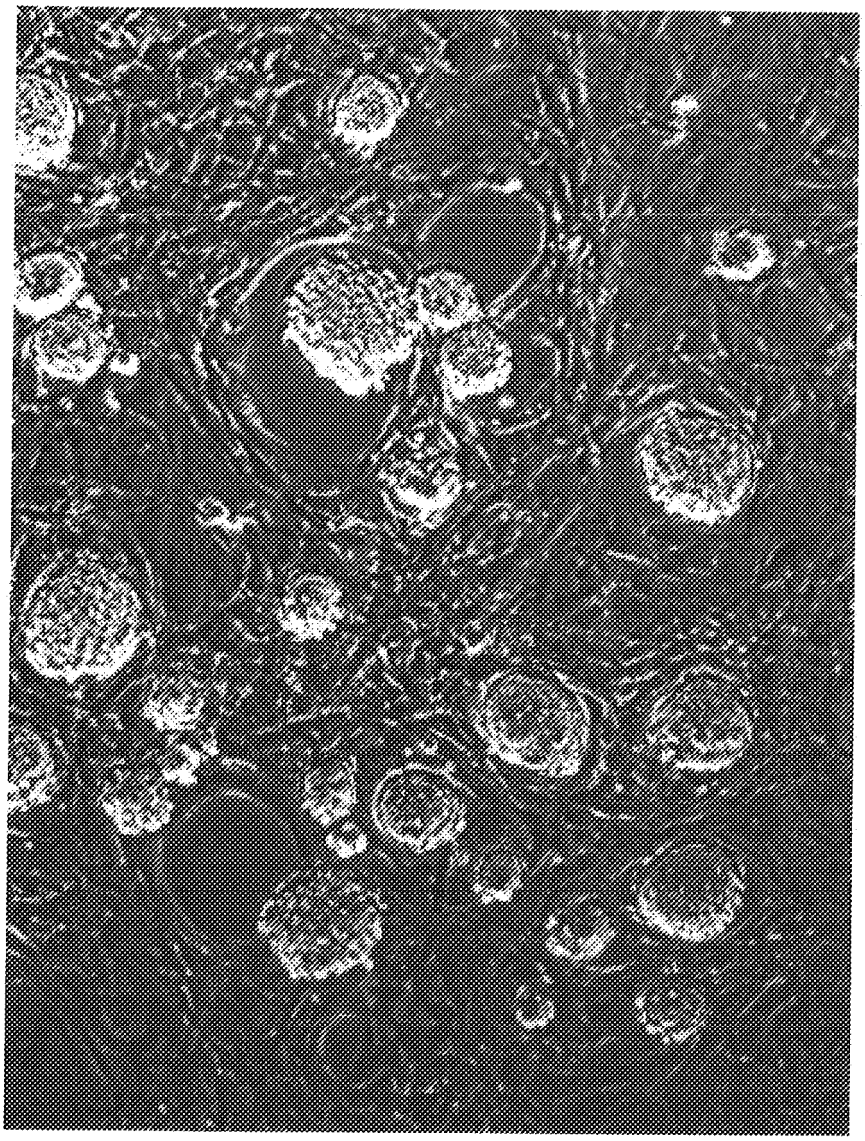


FIG. 1

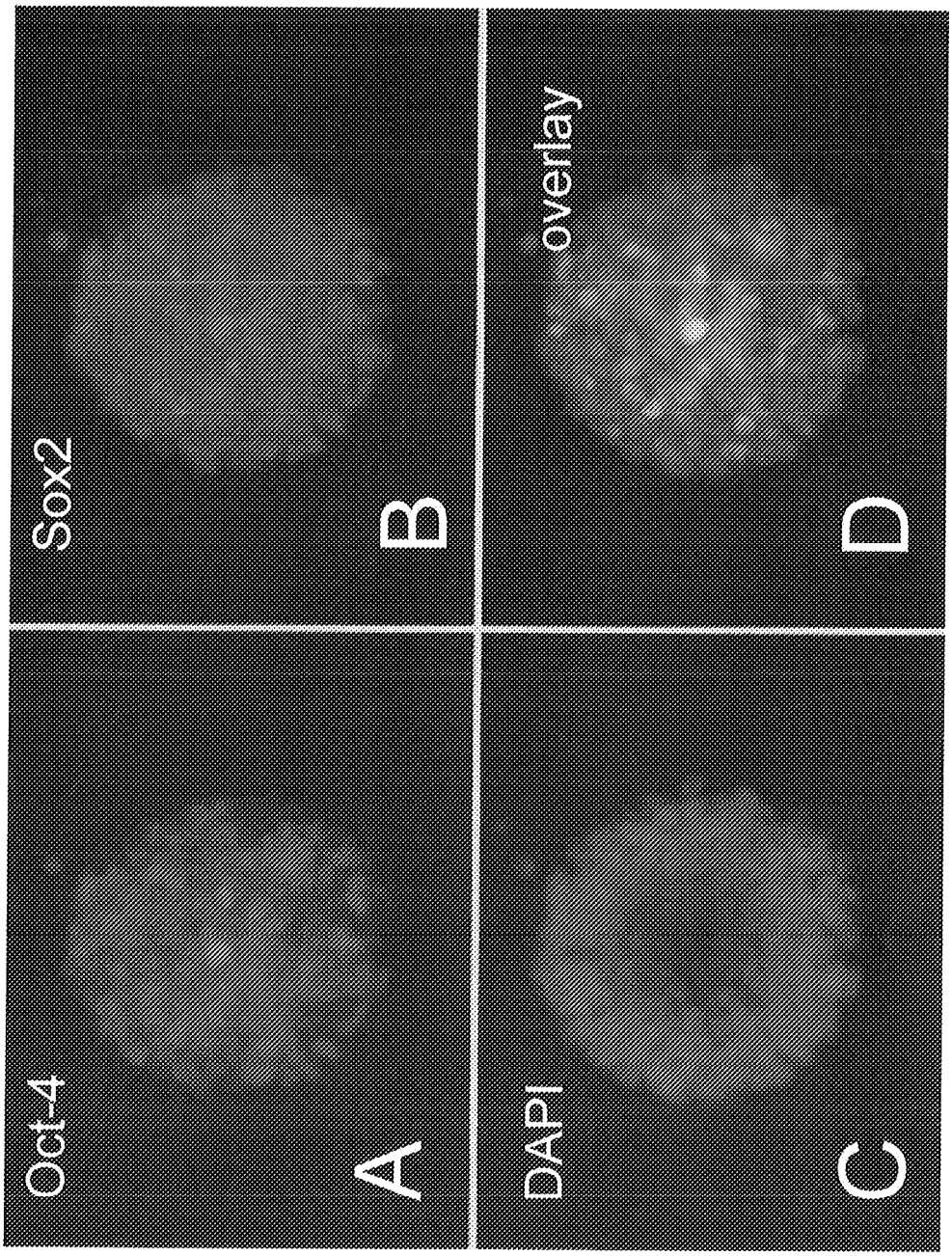


FIG. 2

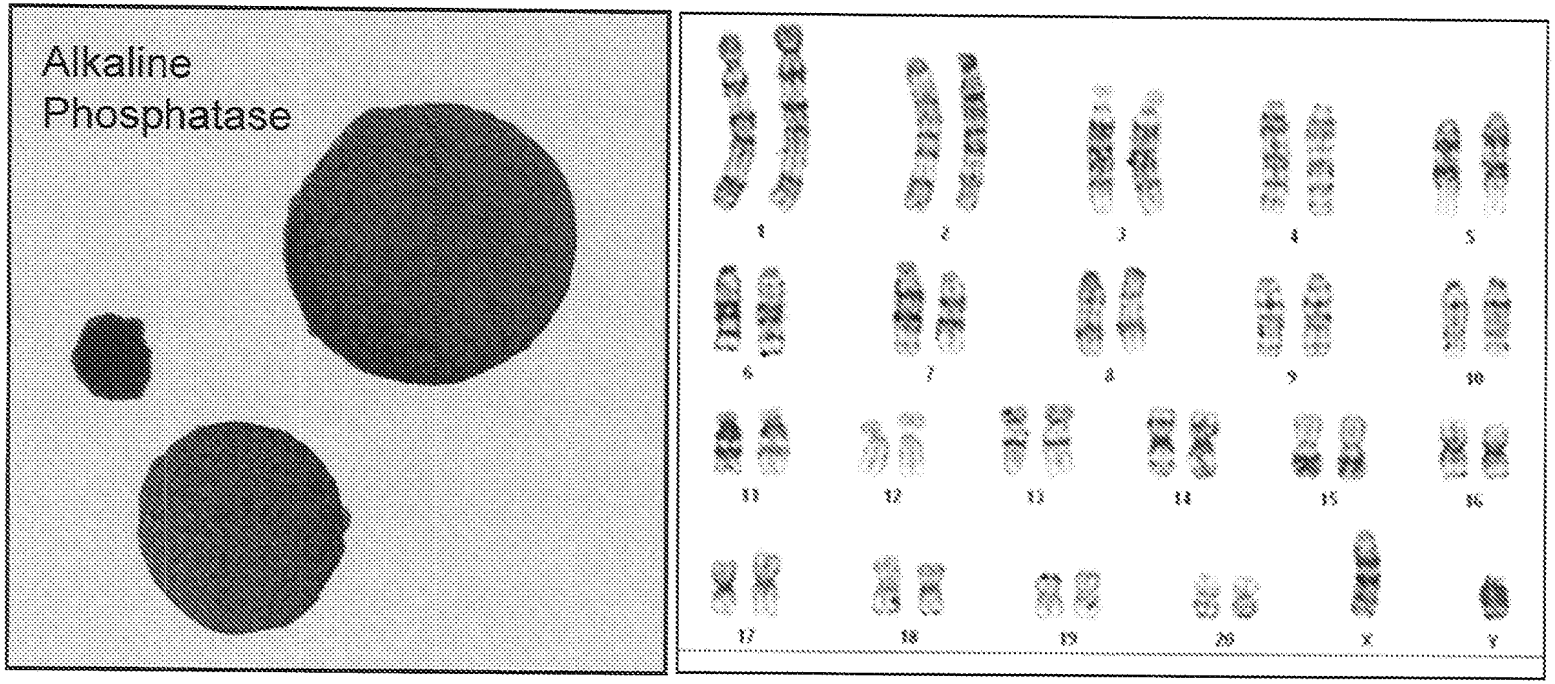


FIG. 3

Cell Results: *42,XX*

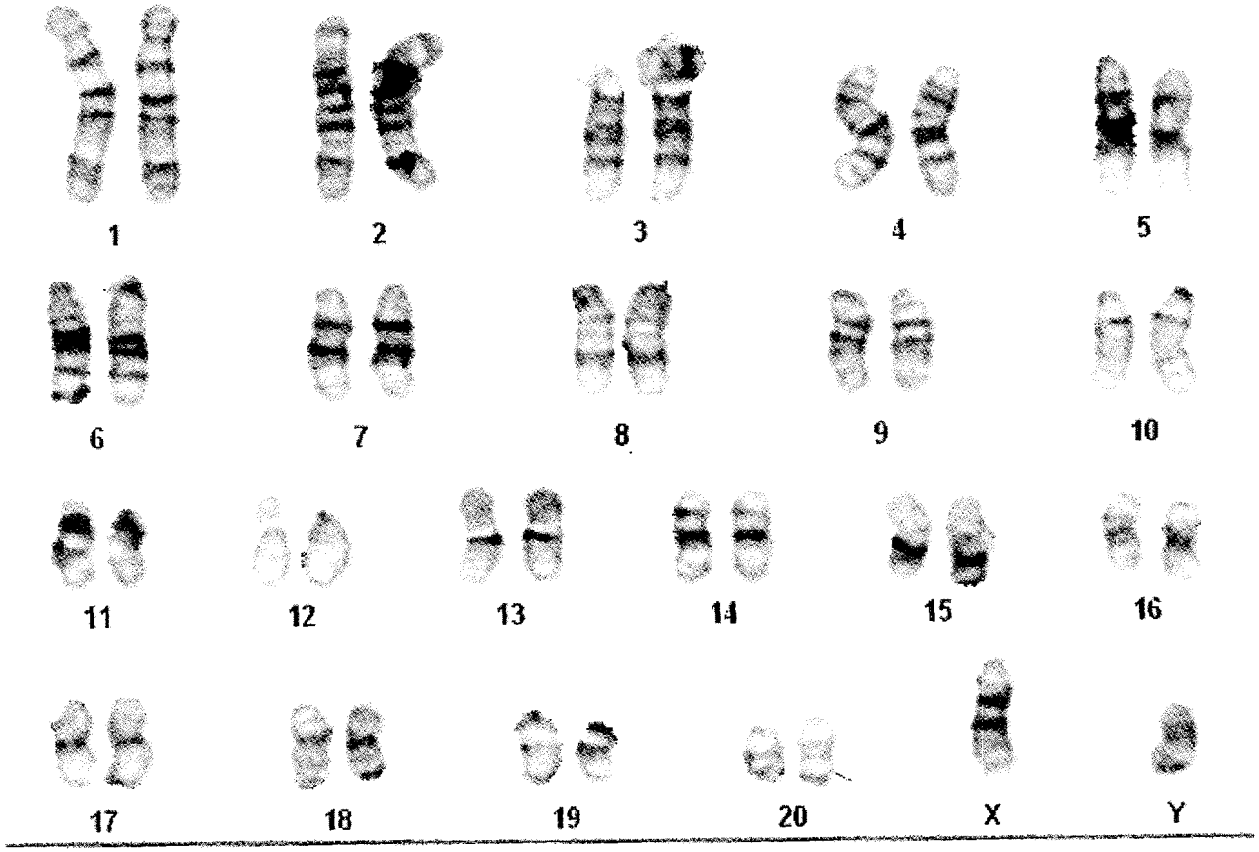


Label - Slide/Cell *met-4 - S01-01*

X, Y: ,

FIG. 4a

Cell Results: *42,XY*

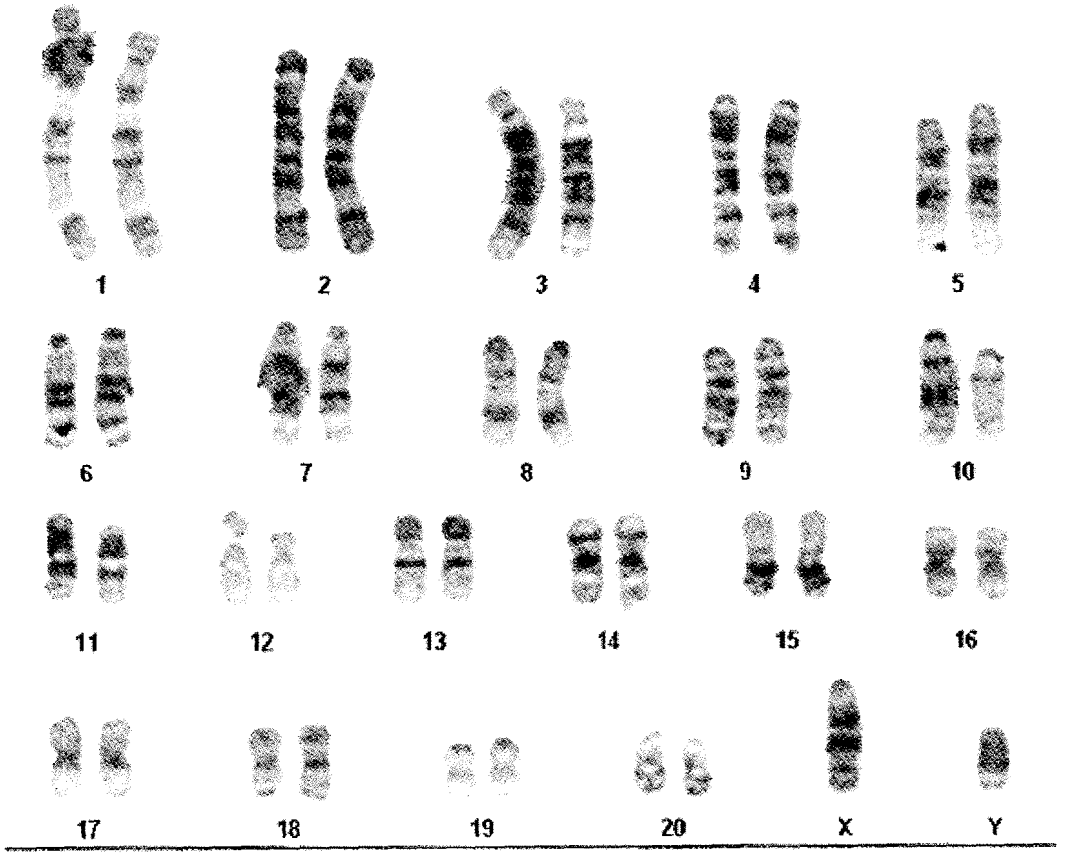


Label - Slide/Cell *met-1 - S01-01*

X, Y:

FIG. 4b

Cell Results: *42,XY*

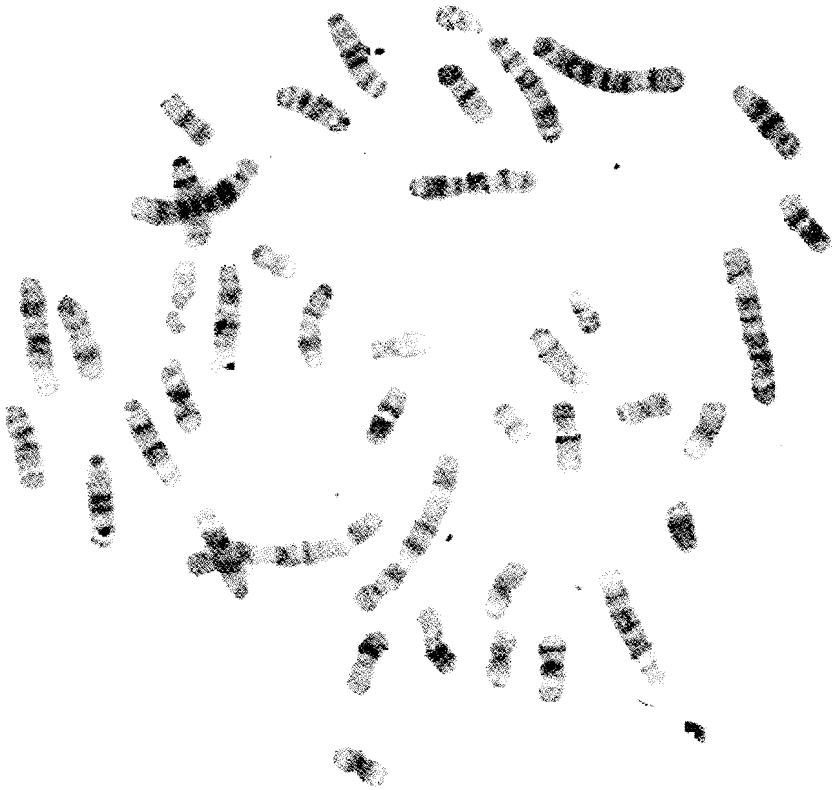


Label - Slide/Cell *met-1 - S01-01*

X, Y:

FIG. 5a

Cell Results: *42,XY*

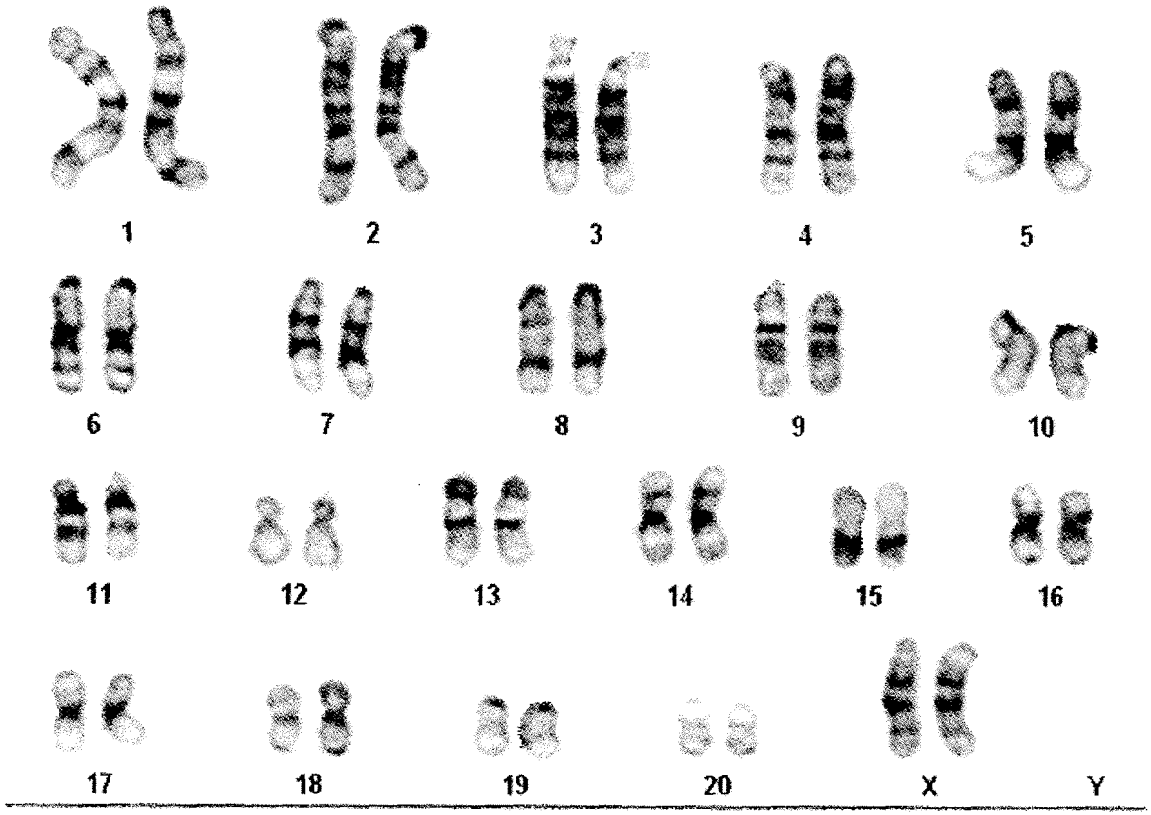


Label - Slide/Cell *met-1 - S01-01*

X, Y:

FIG. 5b

Cell Results: *42,XX*



Label - Slide/Cell *met-4 - S01-01* ***X, Y:***

FIG. 6a

Cell Results: *42,XX*

Figure 6b



Label - Slide/Cell *met-4 - S01-01*

X, Y:

FIG. 6b

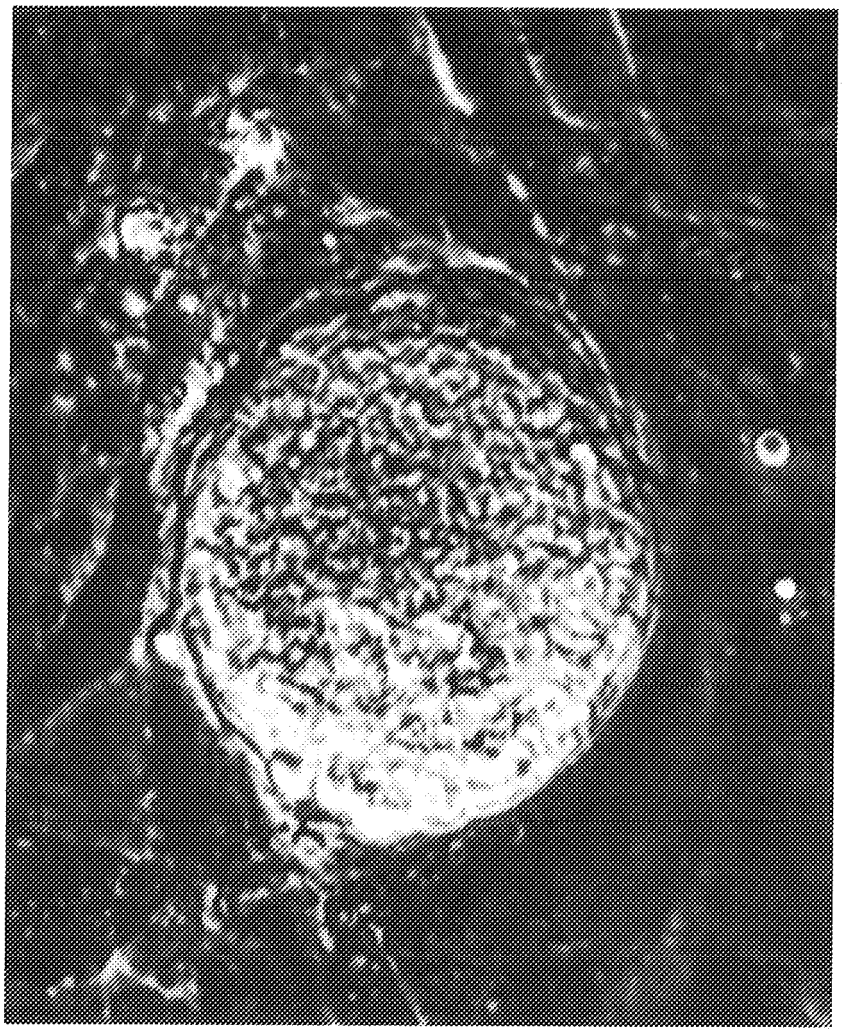


FIG. 7

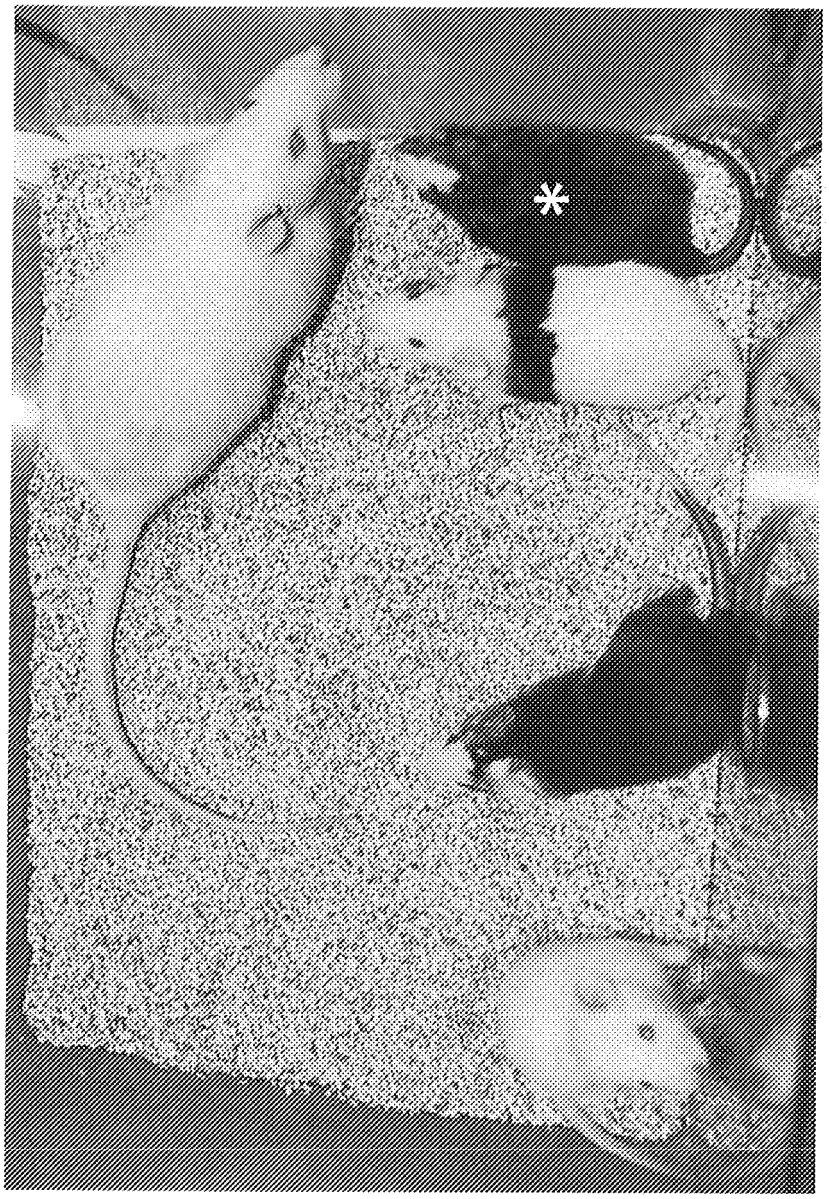


FIG. 8



FIG. 9

Rat ApoE locus and Position of Zinc Finger Cutting Sites

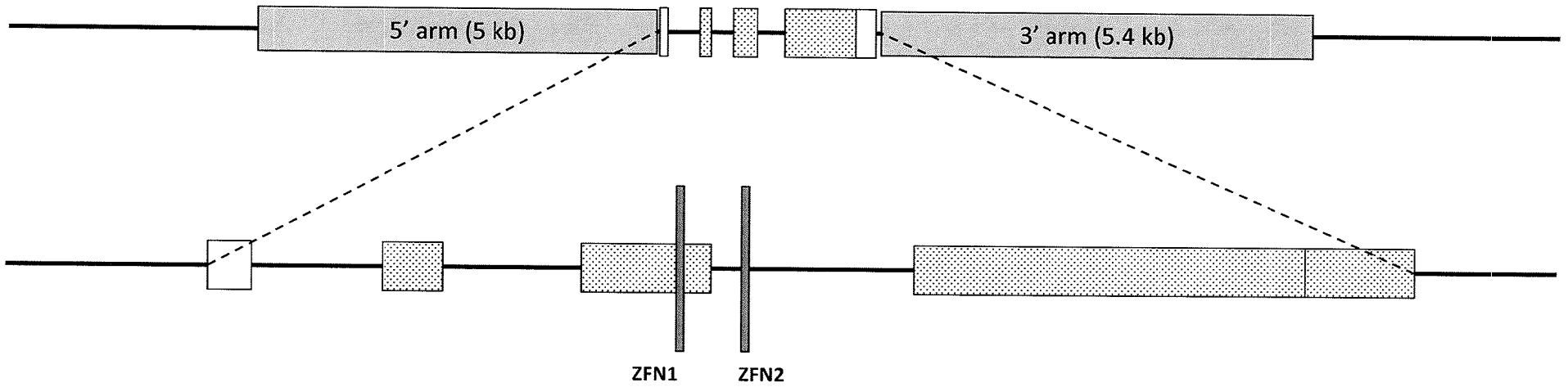


FIG. 10

Rat ApoE ZFNs: improved targeting efficiency

DNA:	Colonies	Screened	Heterozygous Targeted	Homozygous targeted	"Mixed" doubles	Cut/ untargeted
vector	330	184	15 (8.2%)	0	0	N/A
vector + ZFN 1	560	192	**132** (68.8%)	**6** (3.1%)	**18** (9.4%)	**17** (8.9%)
vector + ZFN 2	410	192	**136** (70.8%)	**2** (1.0%)	**6** (3.1%)	**18** (9.4%)

Heterozygous targeted:	1 copy of LOA U & D lost;	1 copy of ZFN probe lost
Mixed doubles:	1 copy of LOA U & D lost;	2 copies of ZFN probe lost
Homozygous targeted:	2 copies of LOA U & D lost;	2 copies of ZFN probe lost

FIG. 11

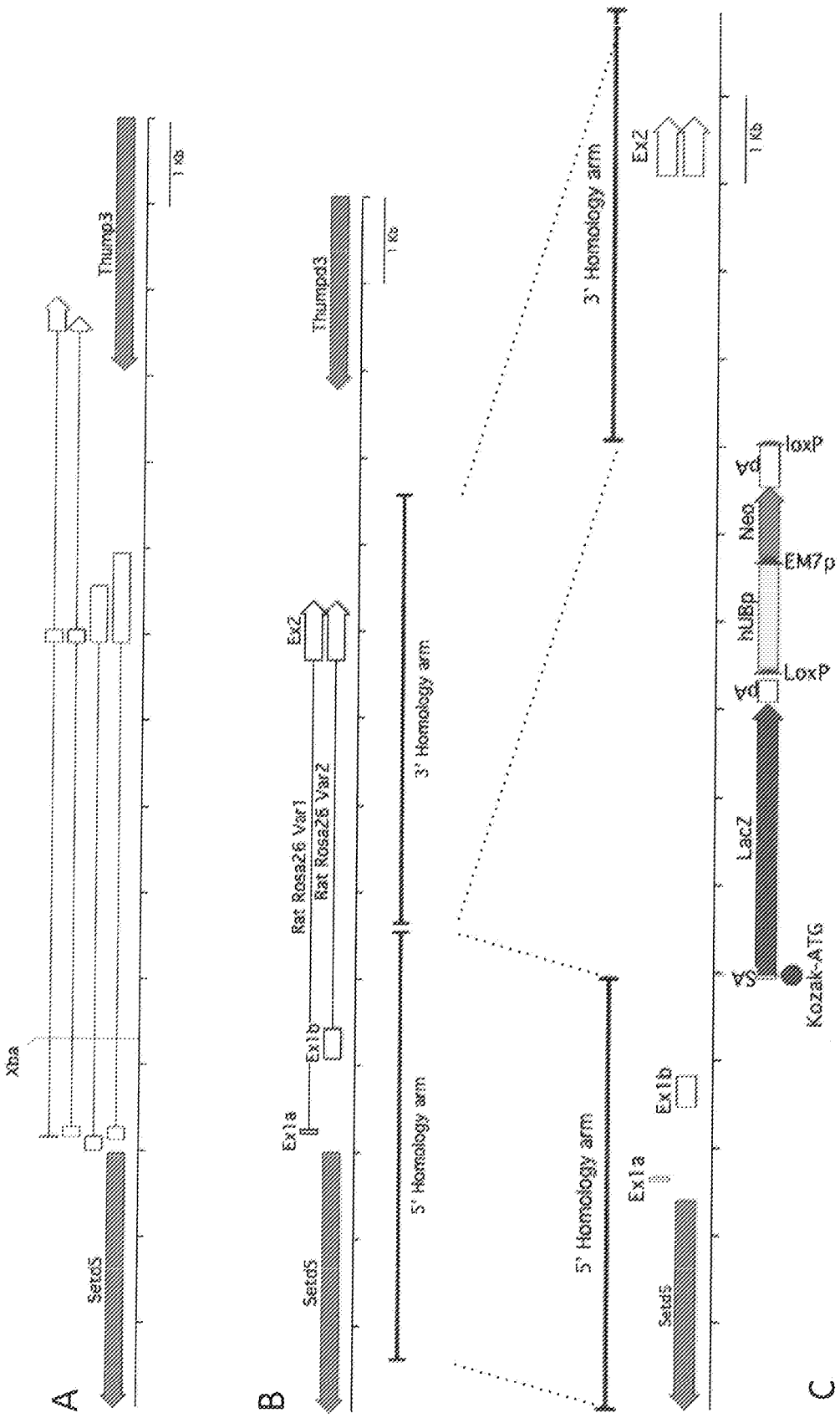

A
Xba
Setd5
Thumpd3
1 Kb
B
Ex1a
Ex1b
Rat Rosa26 Var1
Rat Rosa26 Var2
Ex2
Setd5
Thumpd3
1 Kb
5' Homology arm
3' Homology arm
C
5' Homology arm
3' Homology arm
Ex1a
Ex1b
Ex2
Setd5
1 Kb
LacZ
Kozak-ATG
hUBp
LoxP
Neo
EM7p
loxP

FIG. 12

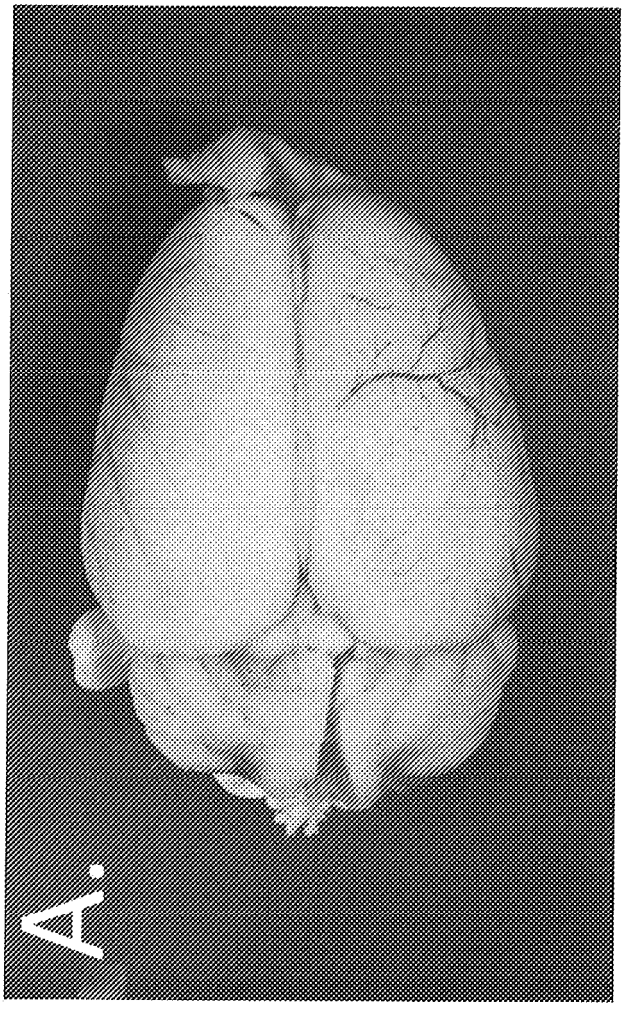


FIG. 13a

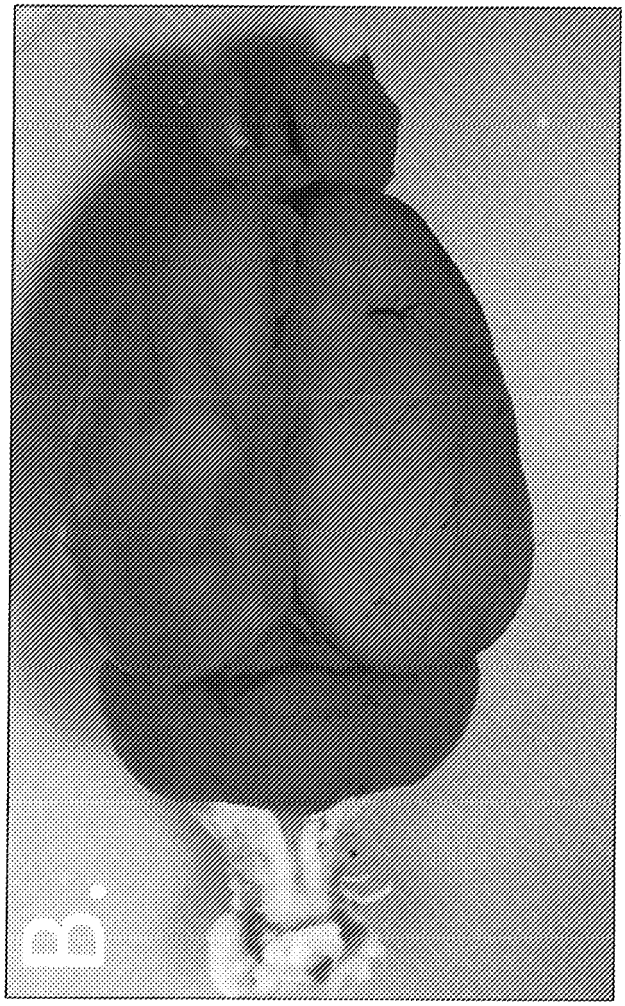


FIG. 13b

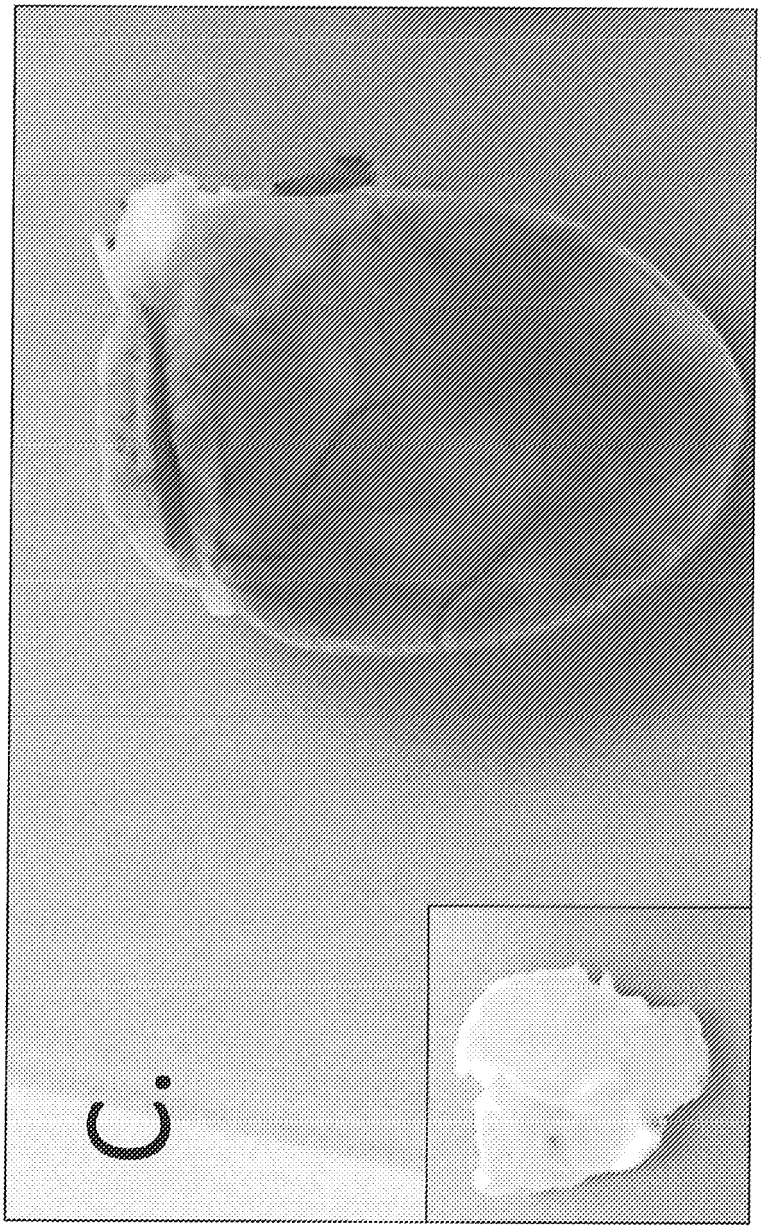


FIG. 13c

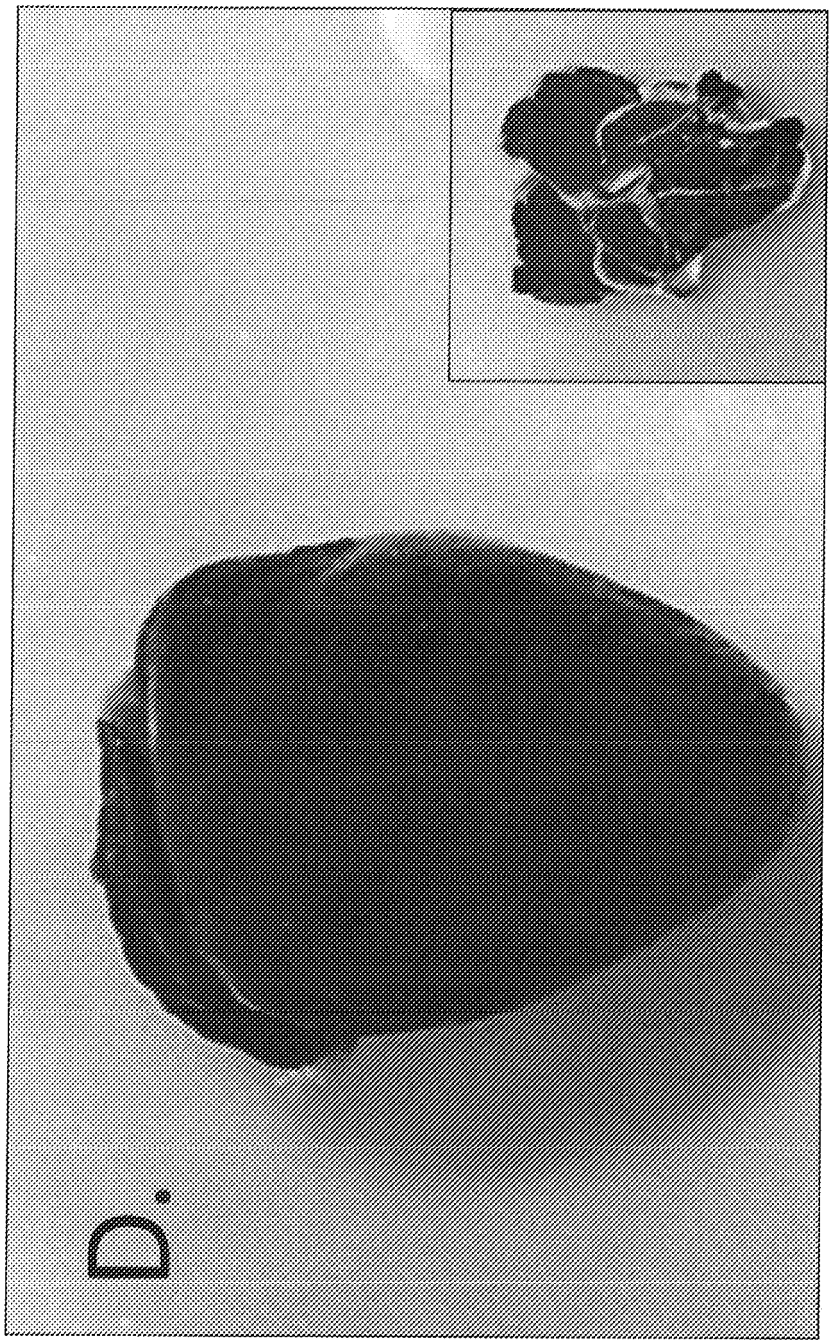


FIG. 13d

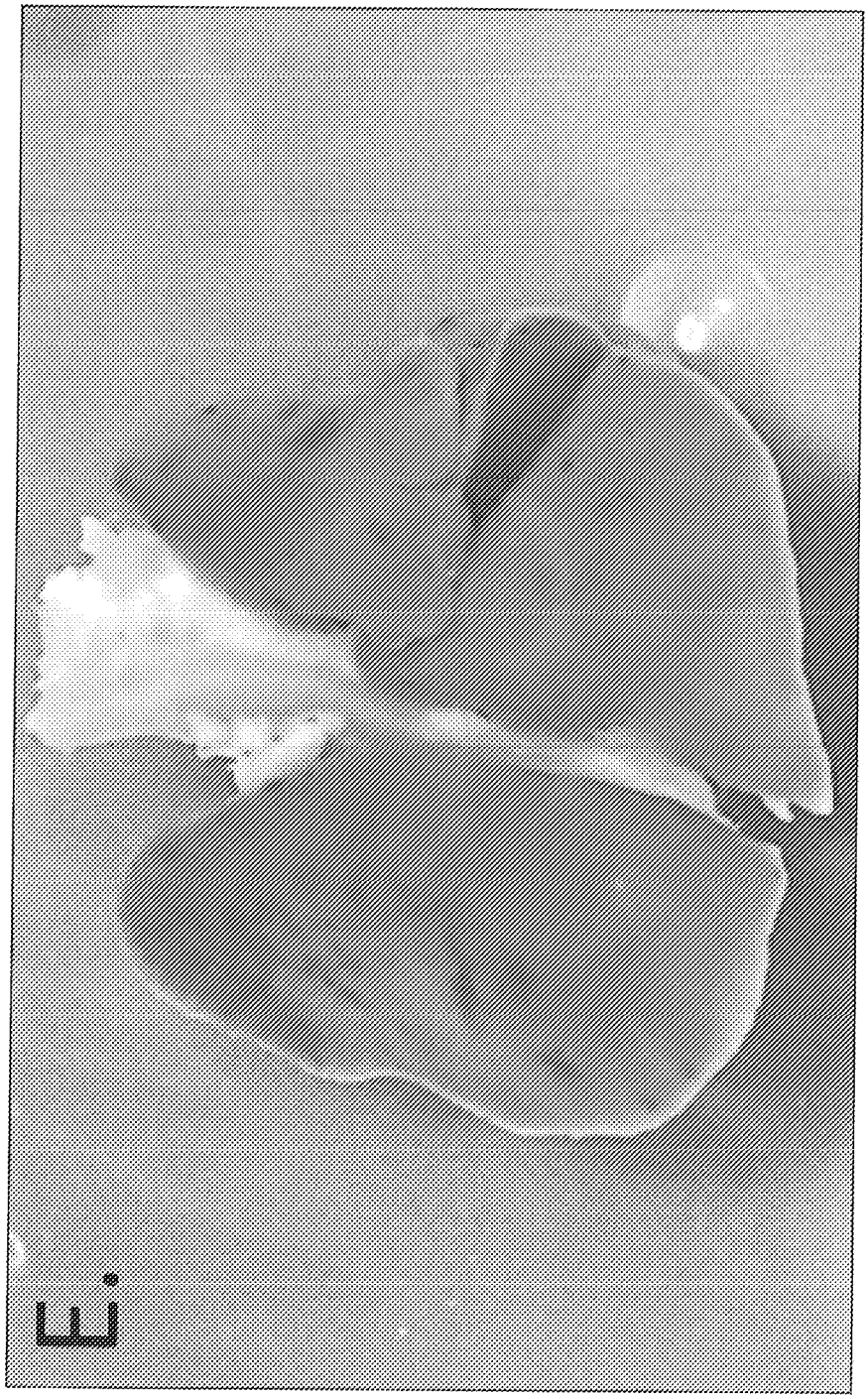


FIG. 13e

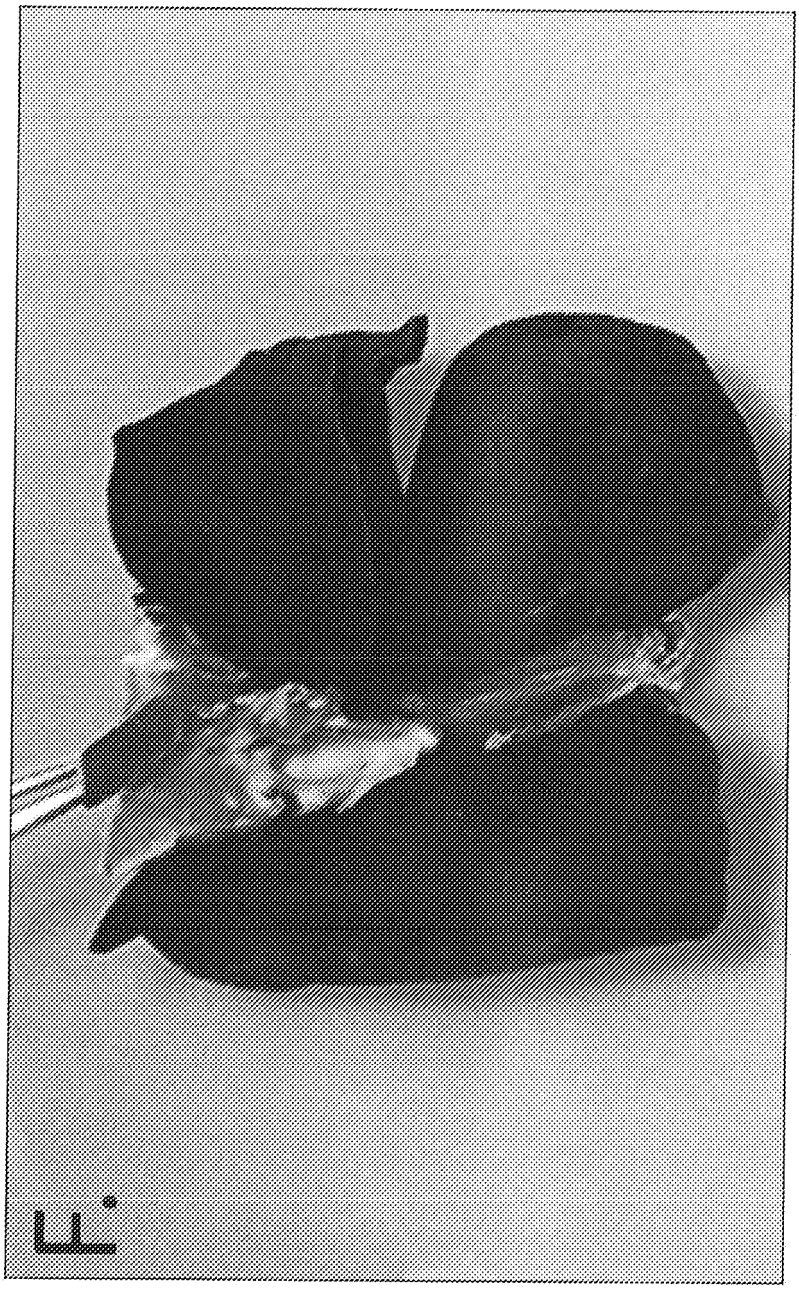


FIG. 13F

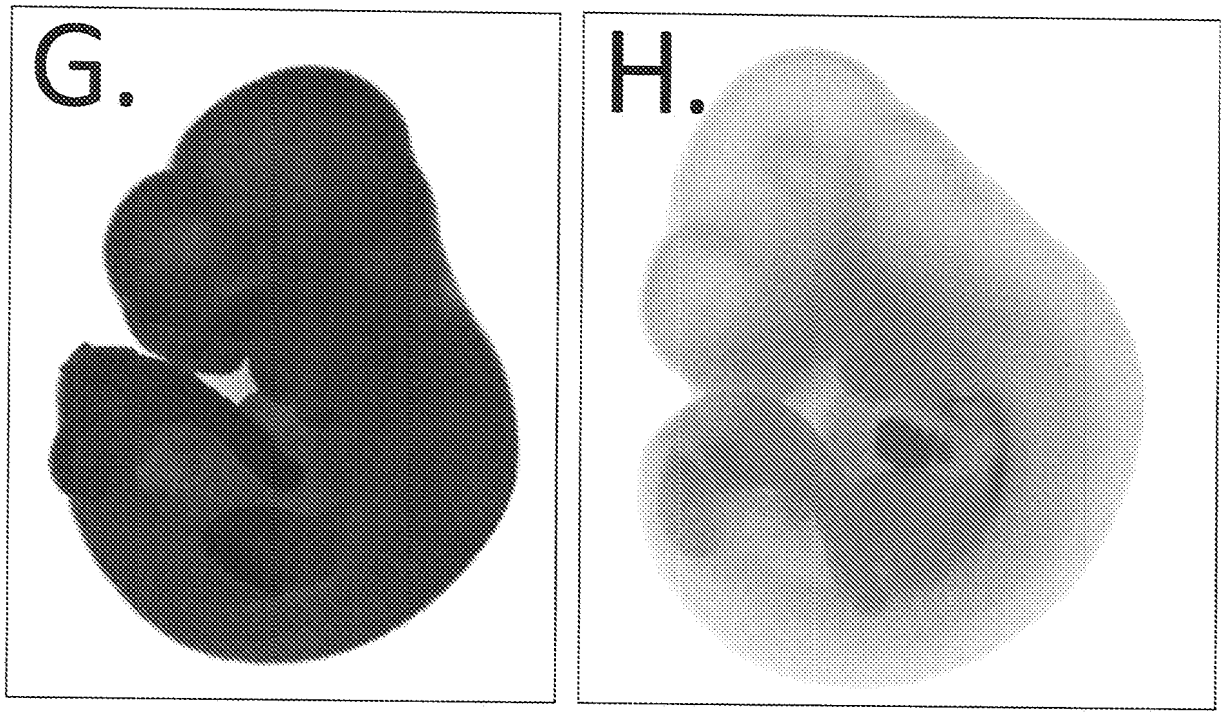


FIG. 13g, h

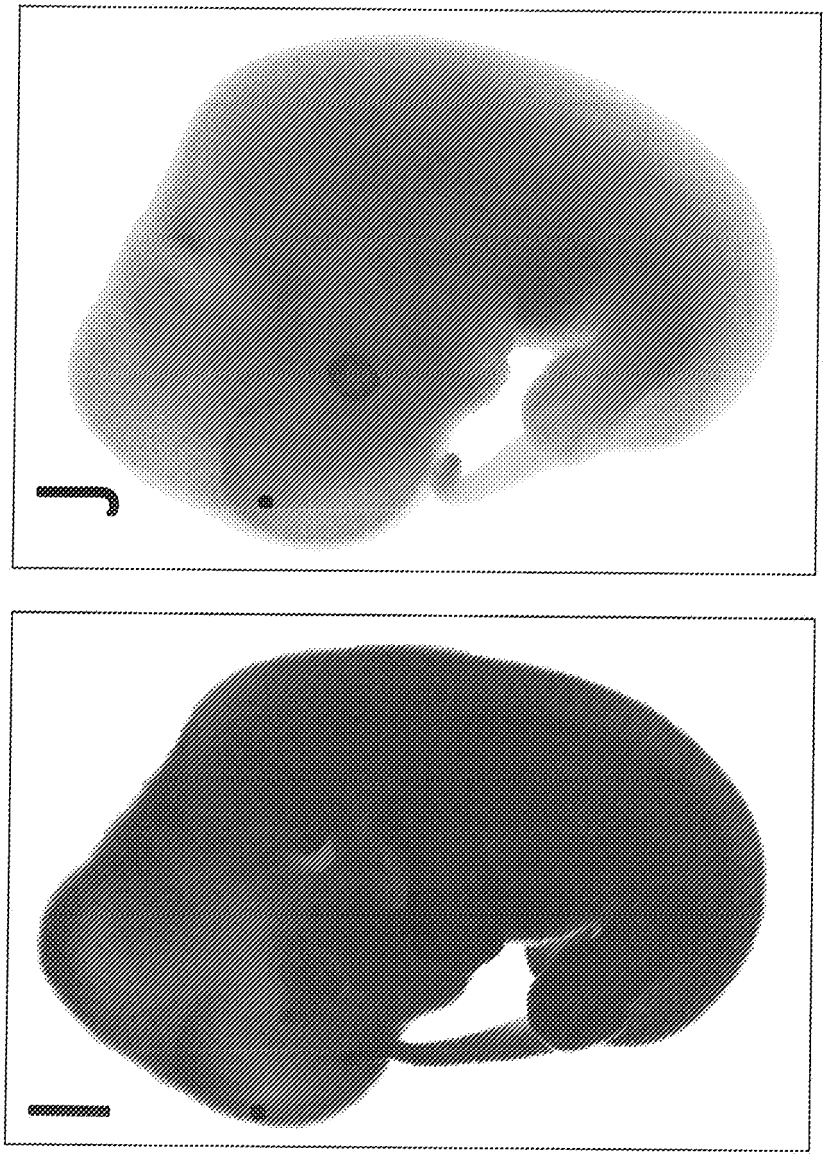


FIG. 13i,j

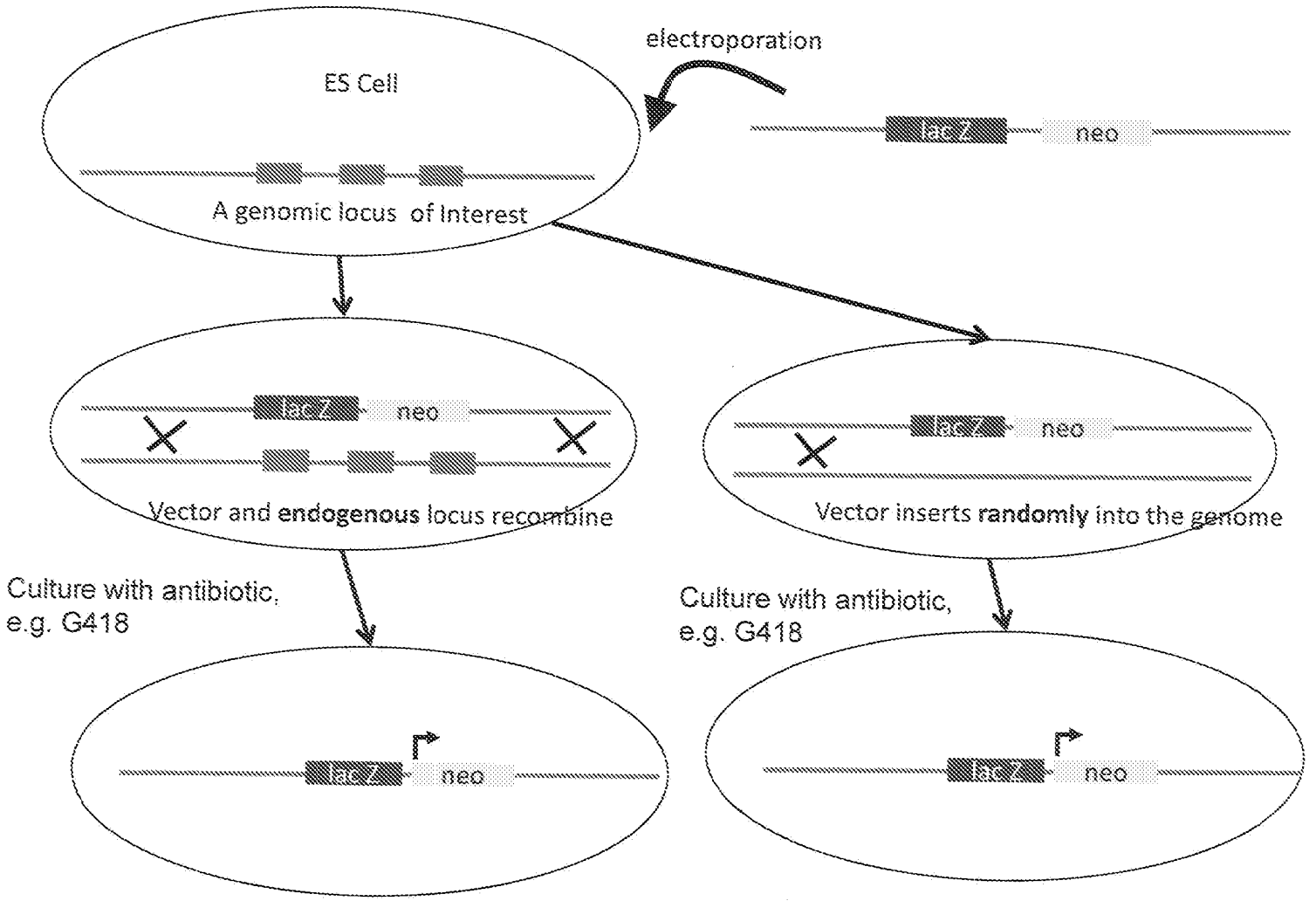

electroporation
ES Cell
A genomic locus of Interest
lac Z
neo
Vector and endogenous locus recombine
Vector inserts randomly into the genome
Culture with antibiotic, e.g. G418
Culture with antibiotic, e.g. G418
Cell is neo^R and grows in presence of G418 typically 1-20% (at best) of insertions
Cell is neo^R and grows in presence of G418

FIG. 14

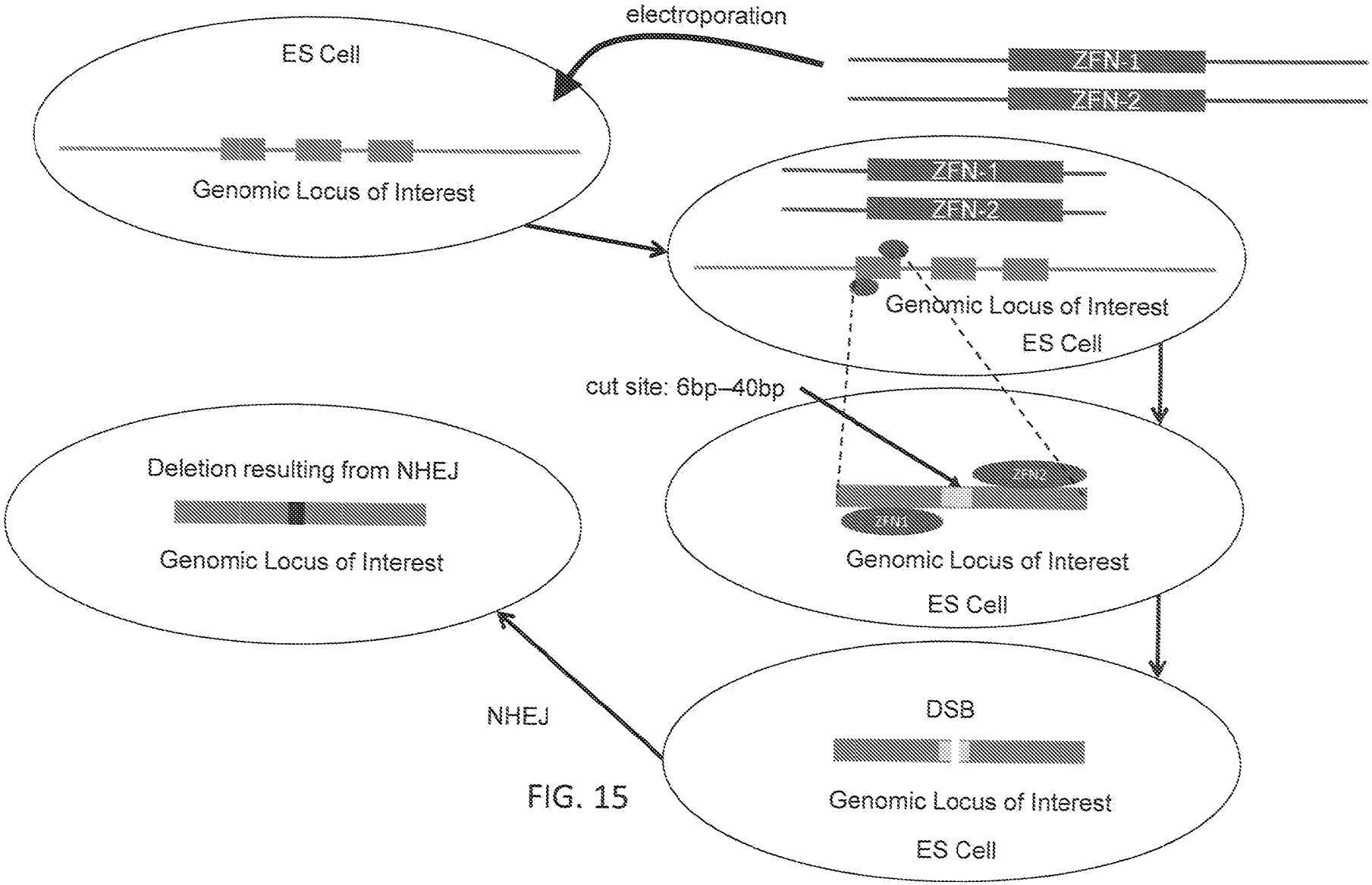

electroporation
ES Cell
Genomic Locus of Interest
ZFN 1
ZFN 2
ZFN 1
ZFN 2
Genomic Locus of Interest
ES Cell
cut site: 6bp–40bp
ZFN 2
ZFN 1
Genomic Locus of Interest
ES Cell
Deletion resulting from NHEJ
Genomic Locus of Interest
NHEJ
DSB
Genomic Locus of Interest
ES Cell

FIG. 15

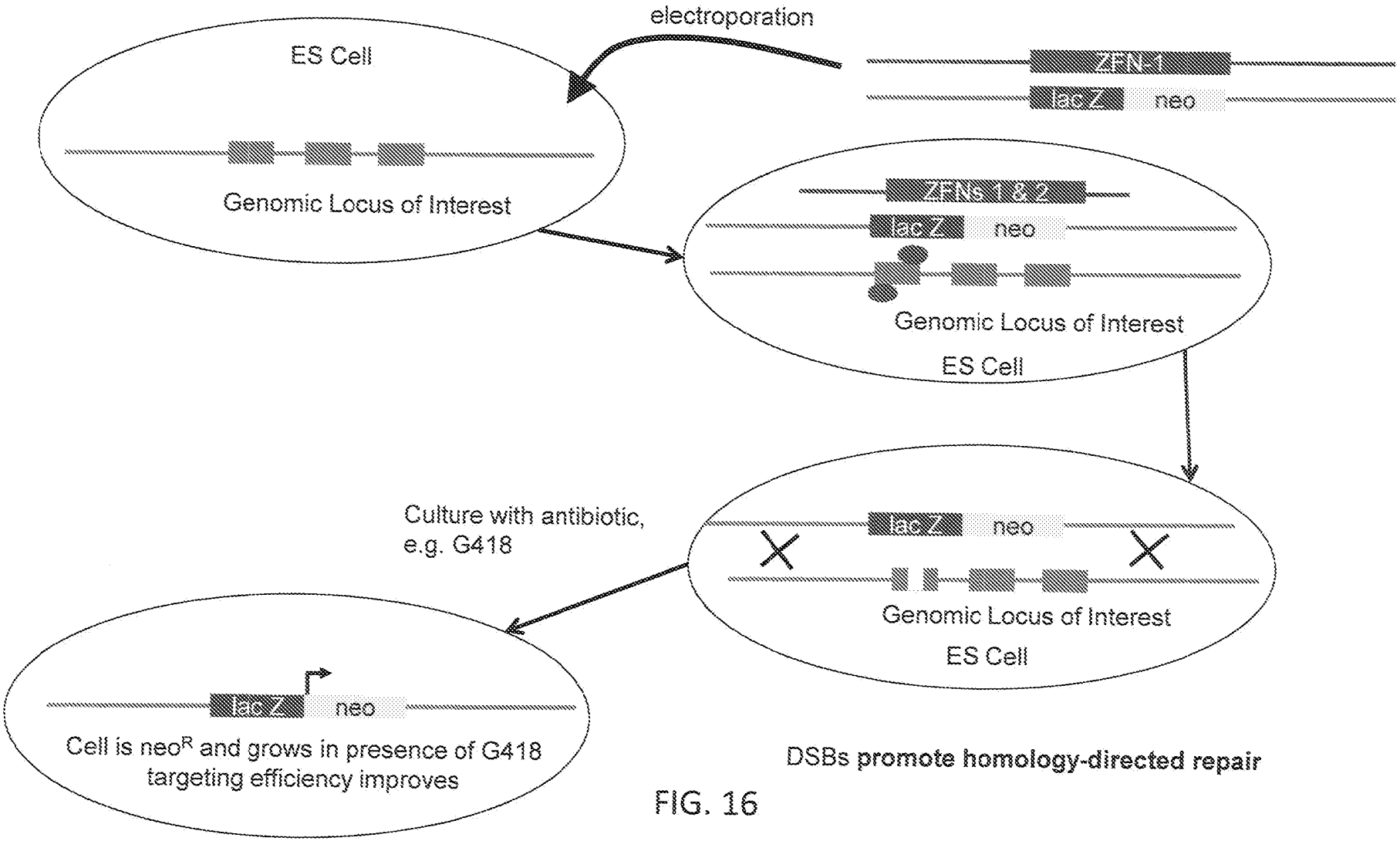

electroporation
ES Cell
Genomic Locus of Interest
ZFNs 1 & 2
lacZ
neo
Genomic Locus of Interest
ES Cell
Culture with antibiotic,
e.g. G418
lacZ
neo
Genomic Locus of Interest
ES Cell
lacZ
neo
Cell is neoR and grows in presence of G418
targeting efficiency improves
DSBs promote homology-directed repair

FIG. 16

Clone		Pups	Chimeras
	ApoE-ZFN1-AB5 (homozyous targeted)	12	4 (90, 90, 80, 80)
	ApoE-ZFN1-AE5 (homozygous targeted)	6	3 (90, 80, 70)



ApoE-ZFN1-AB5 chimeras

FIG. 17

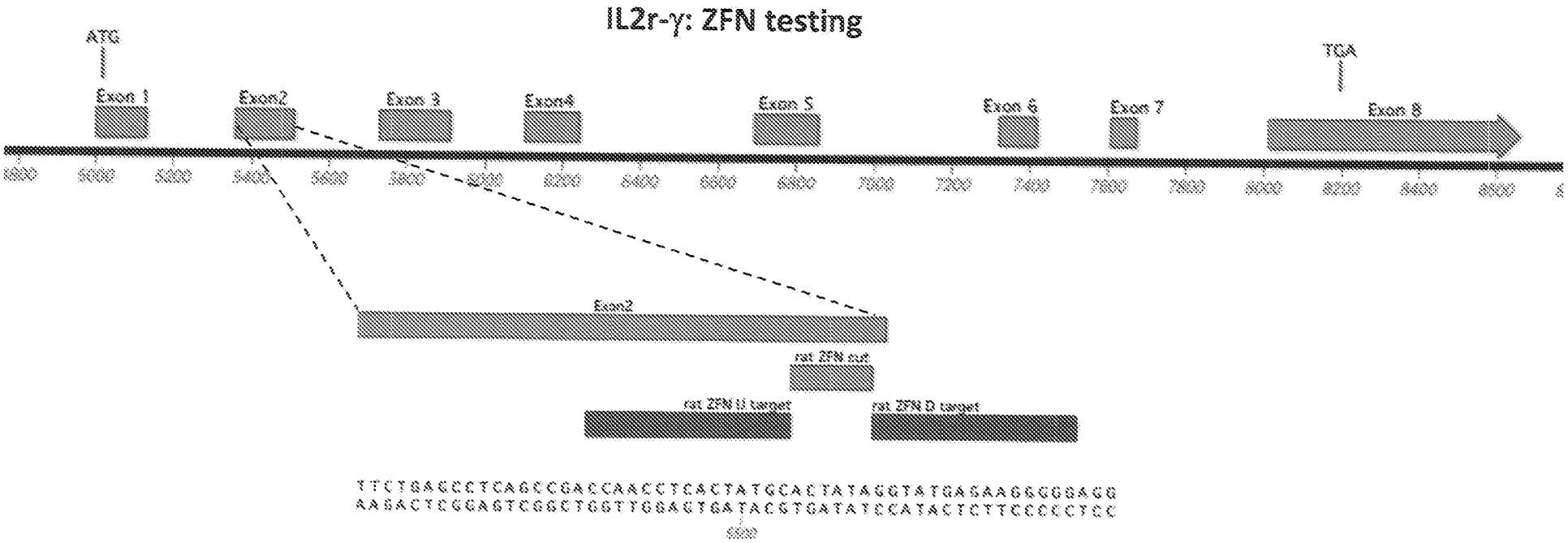
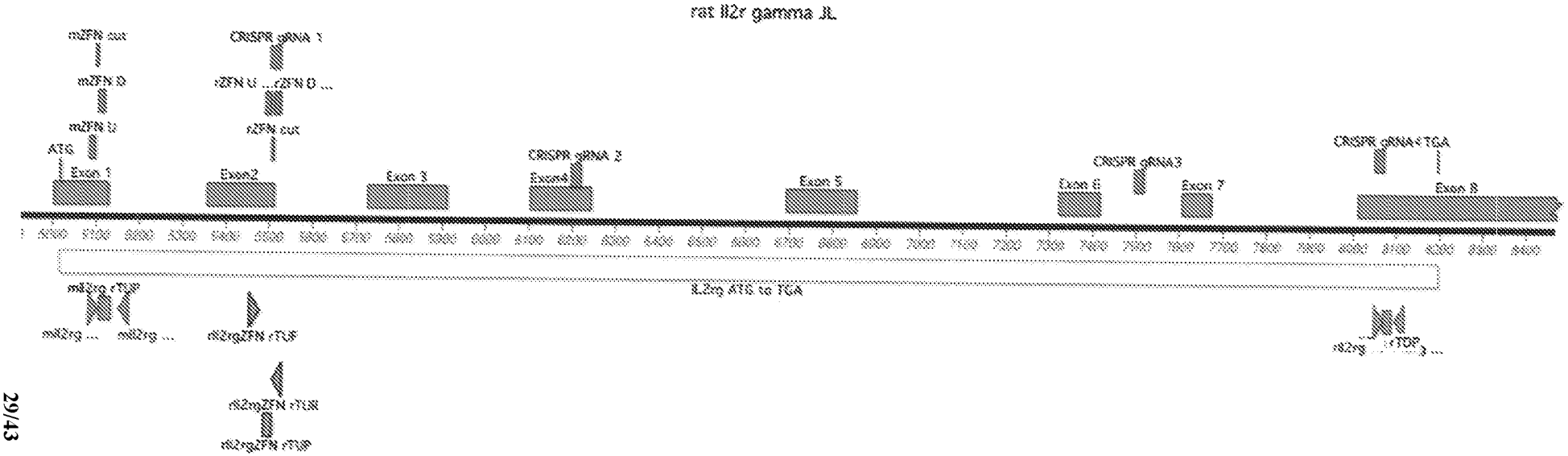


Plate	Colonies screened	Targeted	Efficiency
7/18: Vector alone	96	4	4.2%
7/18: Vector + ZFN	96	3	3.1%

FIG. 18



Construct(s)	DNA (µg)	Colonies	Candidates
Il2rg plasmid vector	6 µg	30	3 (all "weak")
plasmid + SBI gRNA1	6 µg/40 µg	22	1 strong, 1 weak
plasmid + SBI gRNA2	6 µg/40 µg	45	2 strong, 1 weak
plasmid + SBI gRNA3	6 µg/40 µg	66	1 strong, 2 weak
plasmid + SBI gRNA4	6 µg/40 µg	59	0

FIG. 19

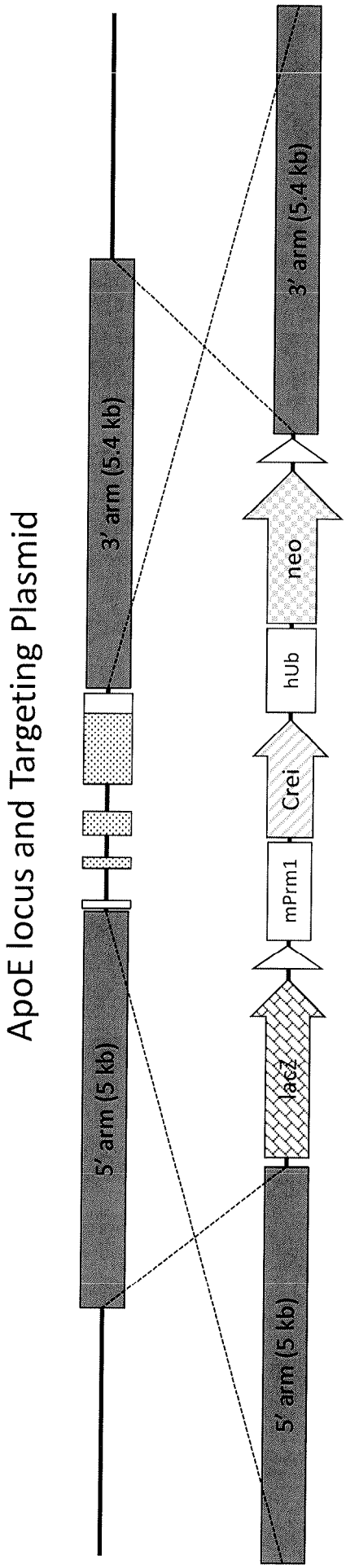
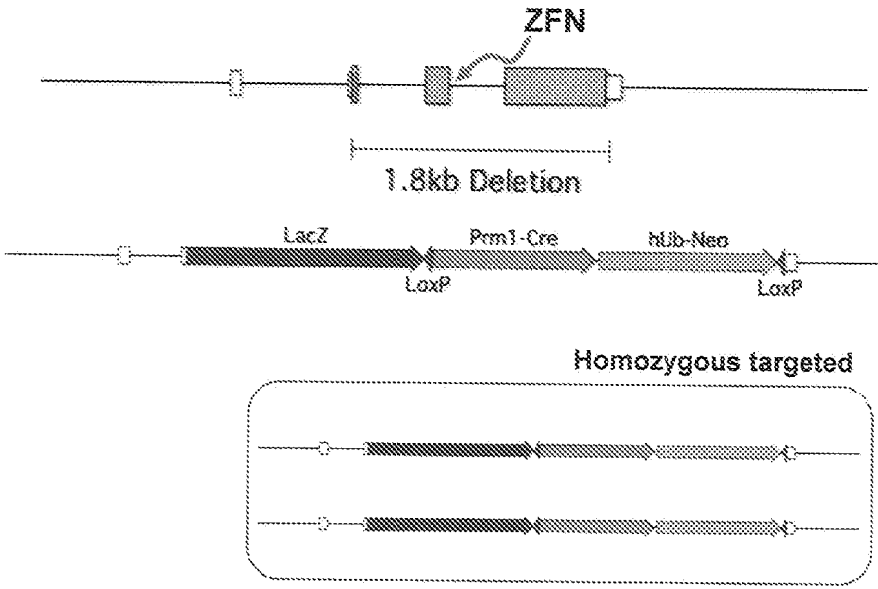

ApoE locus and Targeting Plasmid
3' arm (5.4 kb)
5' arm (5 kb)
lacZ
mPrm1
Crei
hUb
neo
3' arm (5.4 kb)
5' arm (5 kb)

FIG. 20





DNA	Heterozygous targeted	Homozygous Targeted	Microinjected	Chimeras (% chimerism)	Breeding
vector alone	15/192 (8%)	0			
vector + ZFN pair 1	156/192 (81%)	6/192 (3%)	2 clones	7 (70-90%)	
vector + ZFN pair 2	134/192 (70%)	2/192 (1%)			

FIG. 21

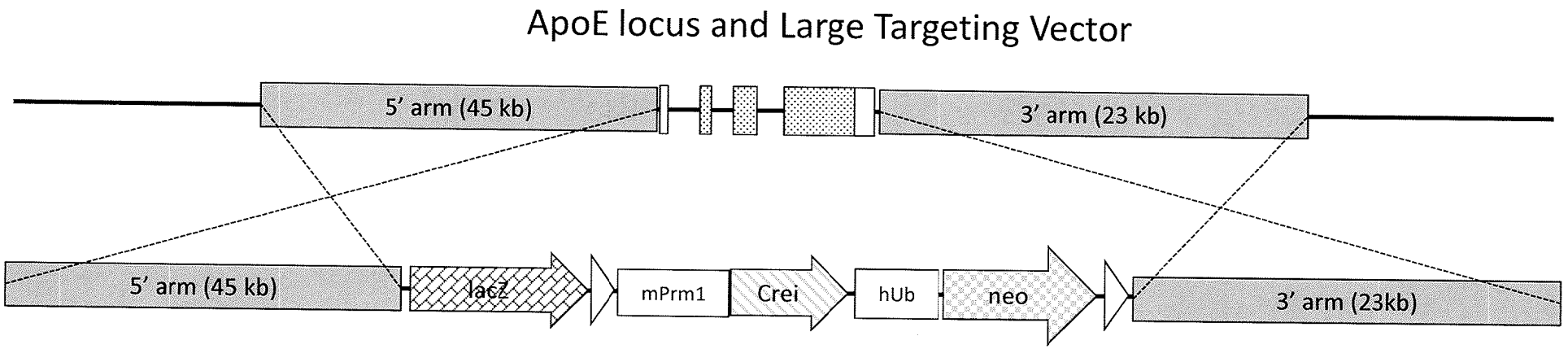

ApoE locus and Large Targeting Vector
5' arm (45 kb)
3' arm (23 kb)
5' arm (45 kb)
lacZ
mPrm1
Crei
hUb
neo
3' arm (23kb)

FIG. 22

Rat ApoE locus and Position of Zinc Finger Cutting Sites

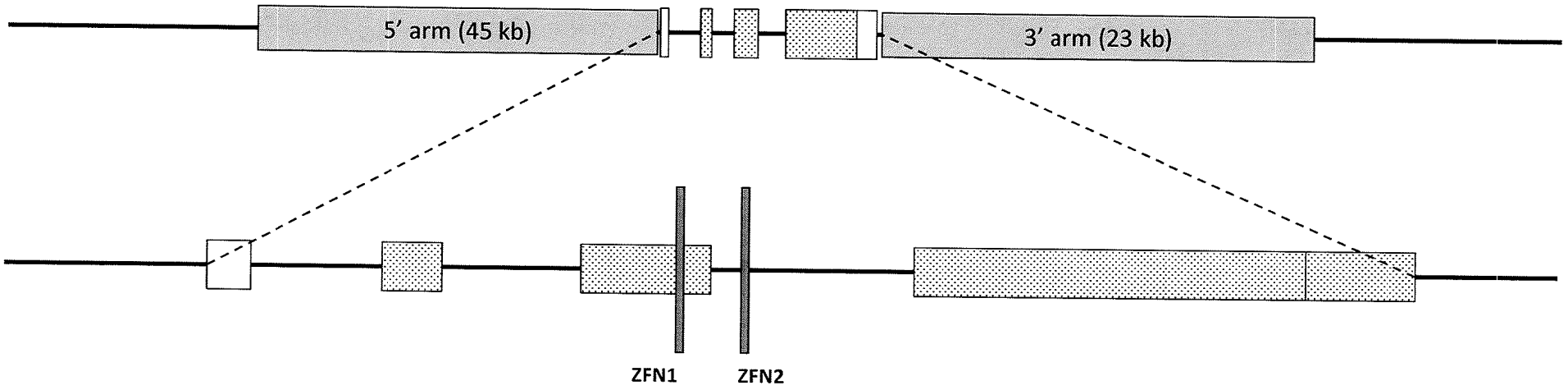


FIG. 23

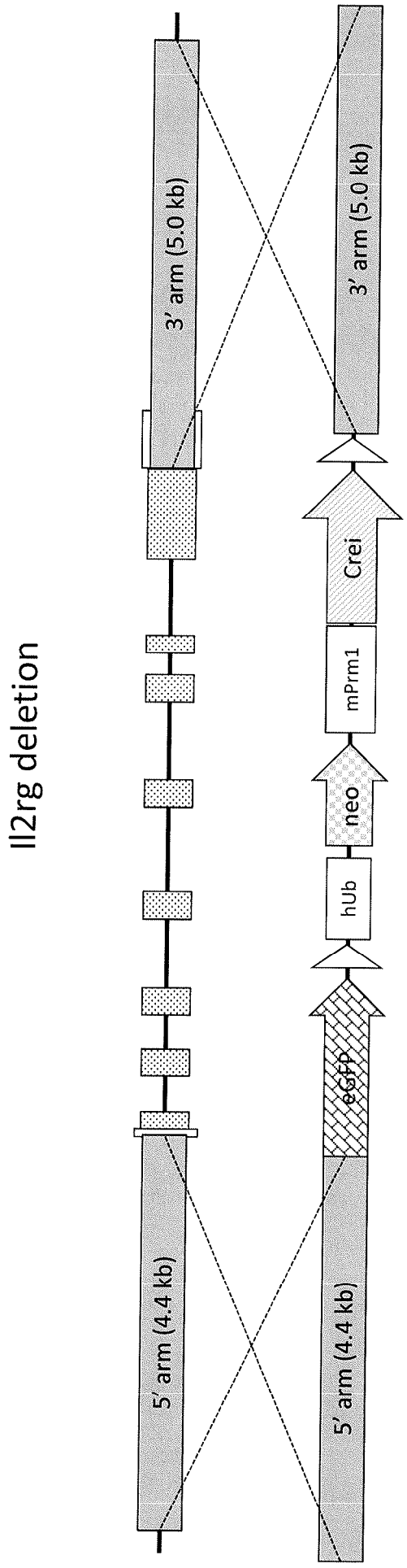

Il2rg deletion
3' arm (5.0 kb)
5' arm (4.4 kb)
eGFP
hUb
neo
mPrm1
Crei
3' arm (5.0 kb)
5' arm (4.4 kb)

FIG. 24

Germline Transmission Confirmed

Line	Karyo-type	Chimeras bred	Germline transmitters	rESC-derived pups	GLT (%)
ACI.G1	XY	5	3 (60%)	11/103	11%
Dark Agouti 2B	XY	5	4 (80%)	11/129	9%
Dark Agouti 2C	XX	3	2 (66%)	7/45	16%

Cell line	Positives	Targeting efficiency
DA.2B	8/192	4.2%
ACI.G1	4/192	2.1%

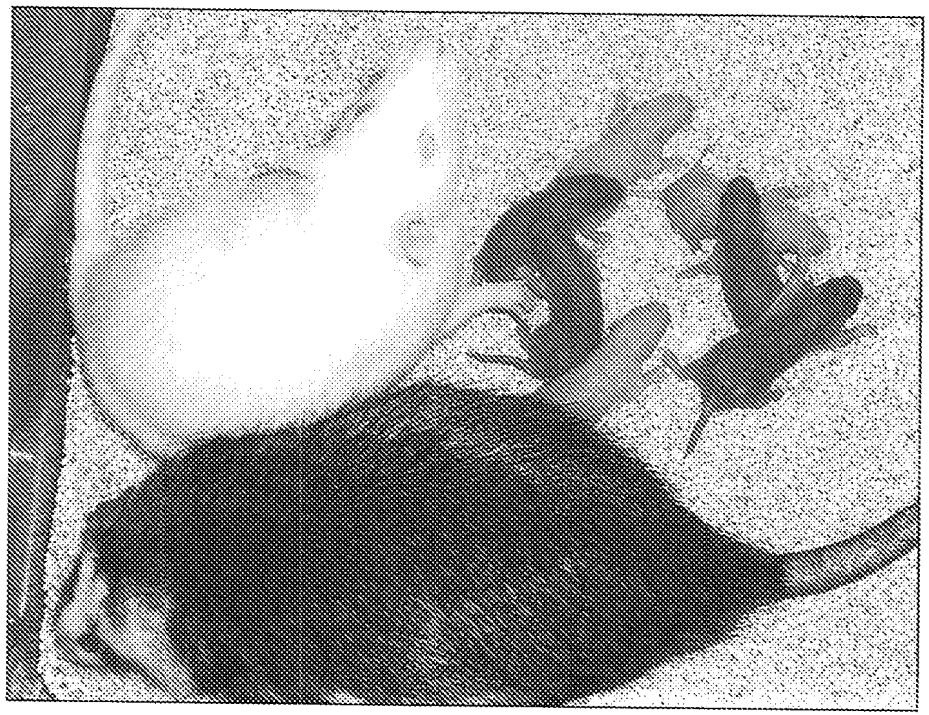
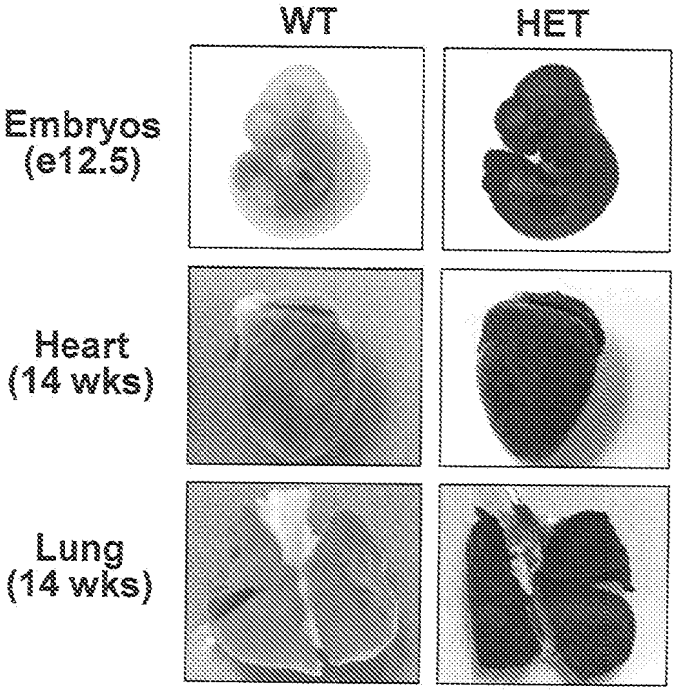
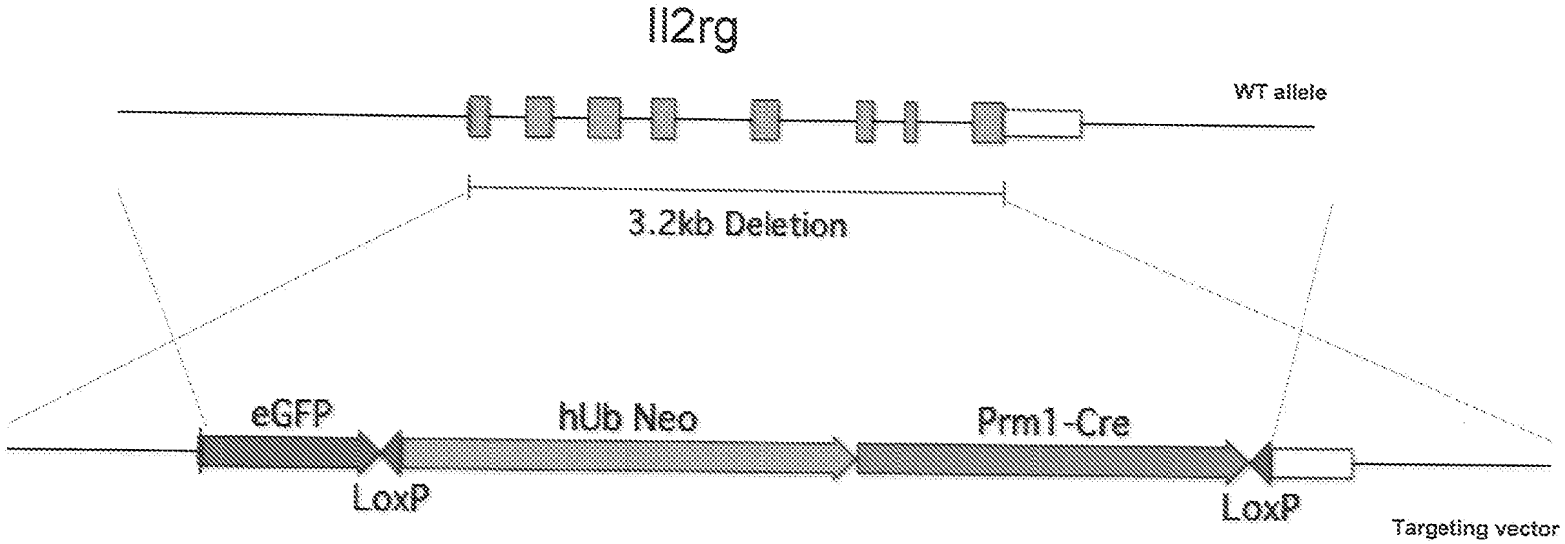


FIG. 25





Gene	Targeted Clones		Chimeras (% chimerism)	
Il2rg	4/232 (2%)		5 (70 – 90%)	

FIG. 26

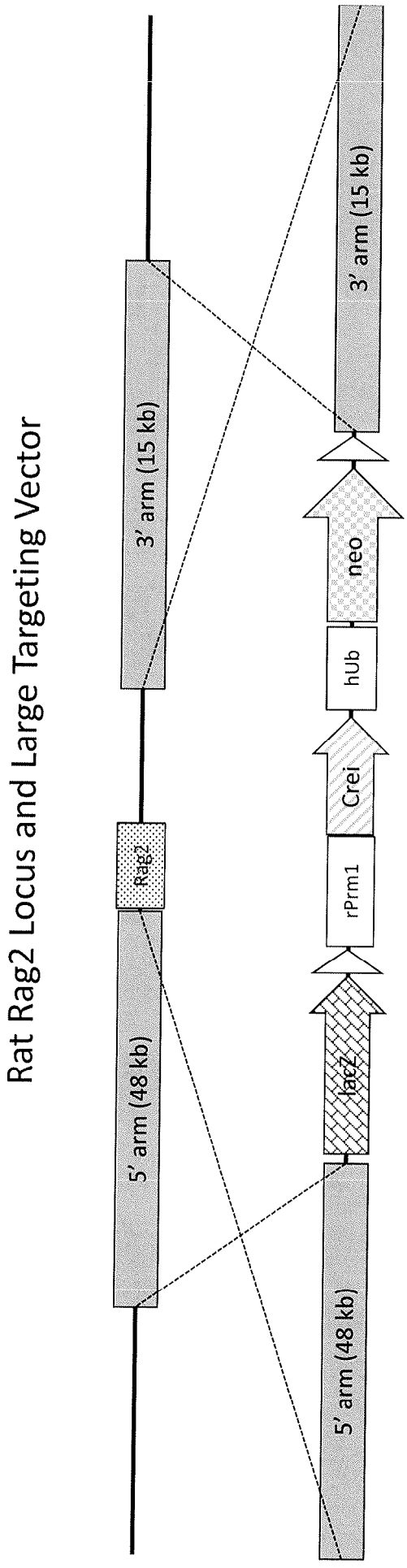

Rat Rag2 Locus and Large Targeting Vector
3' arm (15 kb)
Rag2
5' arm (48 kb)
3' arm (15 kb)
neo
hUb
Crei
rPrm1
lacZ
5' arm (48 kb)

FIG. 27

Rag2/Rag1 targeting

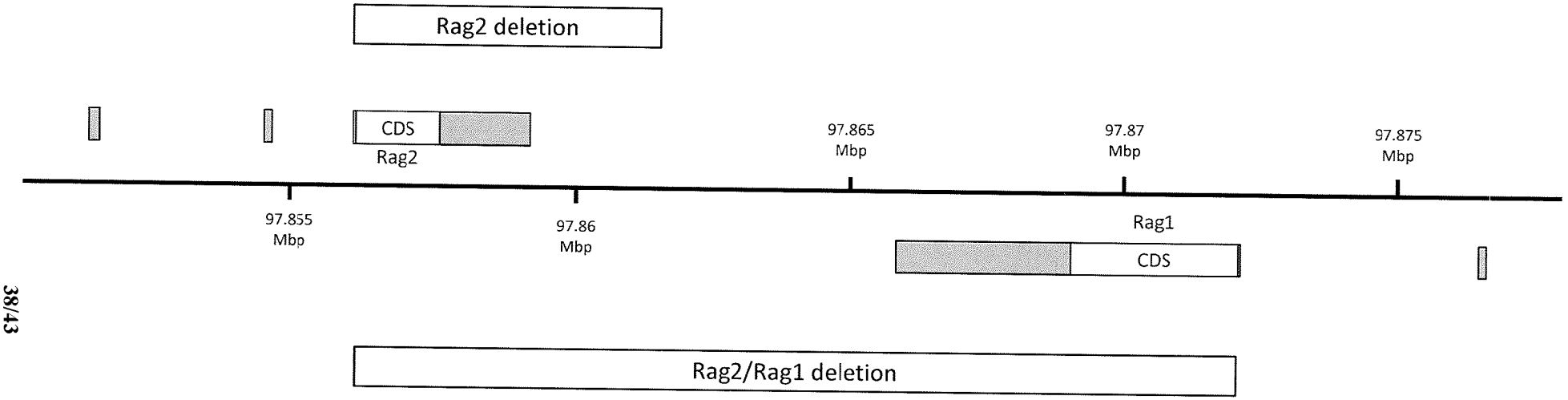


FIG. 28

Rag2/Rag1 targeting

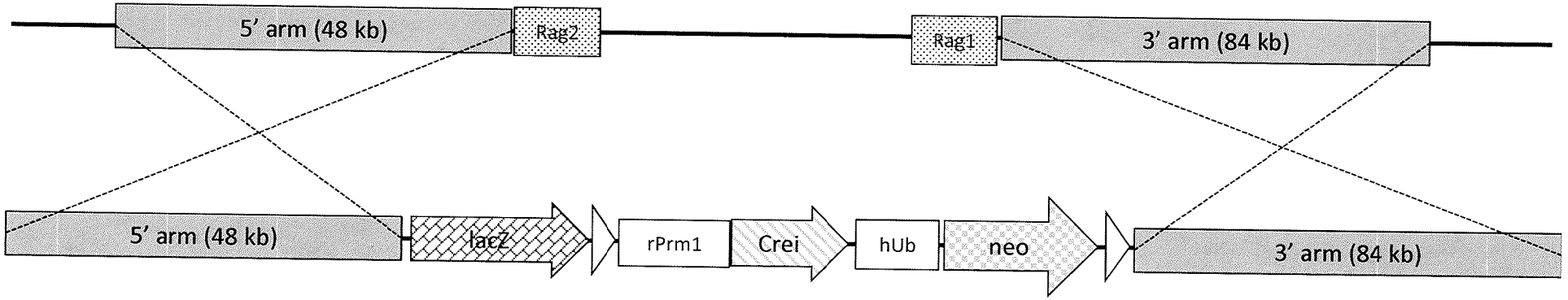


FIG. 29

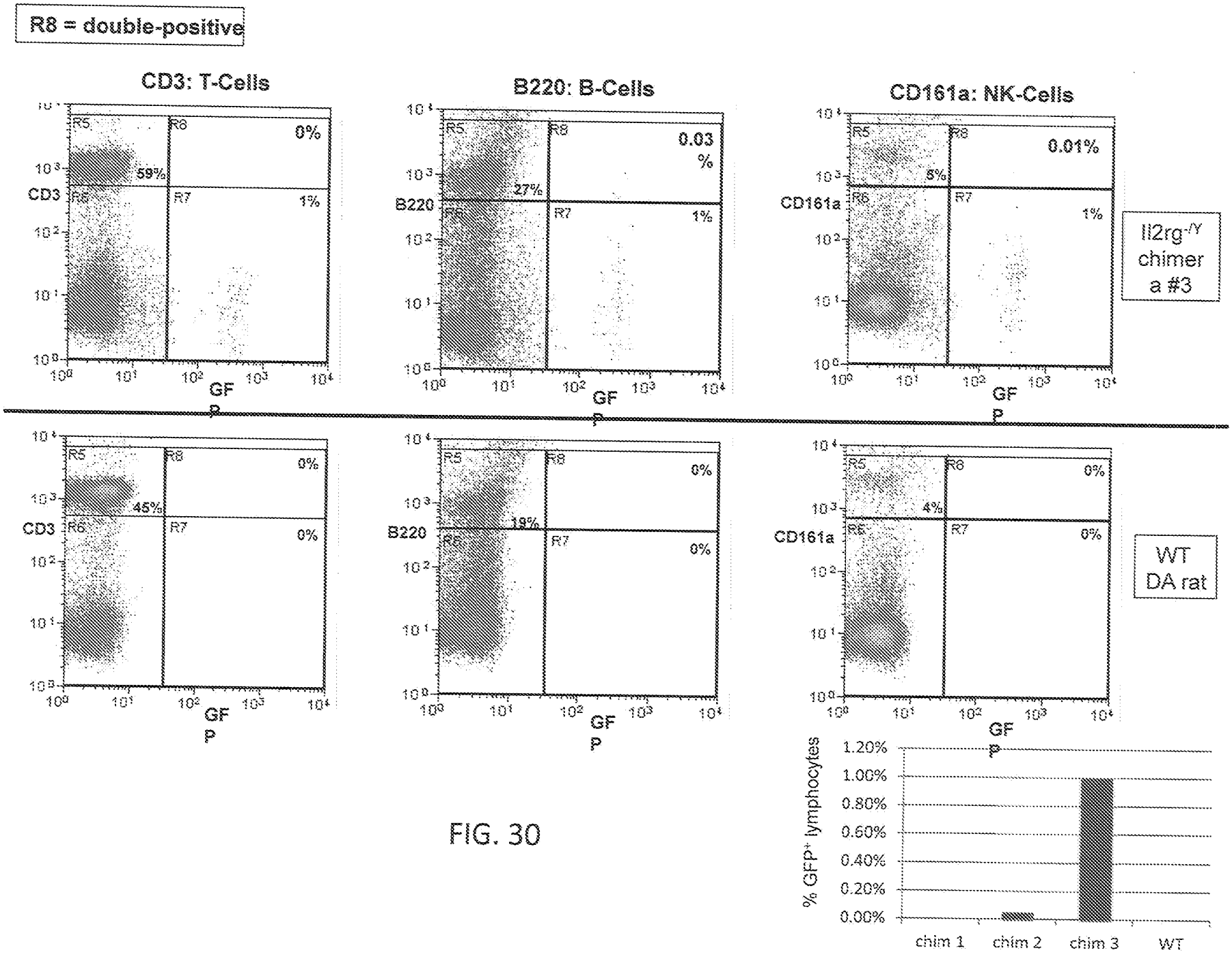

R8 = double-positive
CD3: T-Cells
B220: B-Cells
CD161a: NK-Cells
R5
R6
R7
R8
0%
59%
1%
0.03
%
27%
1%
0.01%
5%
1%
Il2rg-/Y
chimer
a #3
45%
0%
0%
19%
0%
4%
0%
WT
DA rat
CD3
B220
CD161a
GFP
% GFP+ lymphocytes
1.20%
1.00%
0.80%
0.60%
0.40%
0.20%
0.00%
chim 1
chim 2
chim 3
WT

FIG. 30

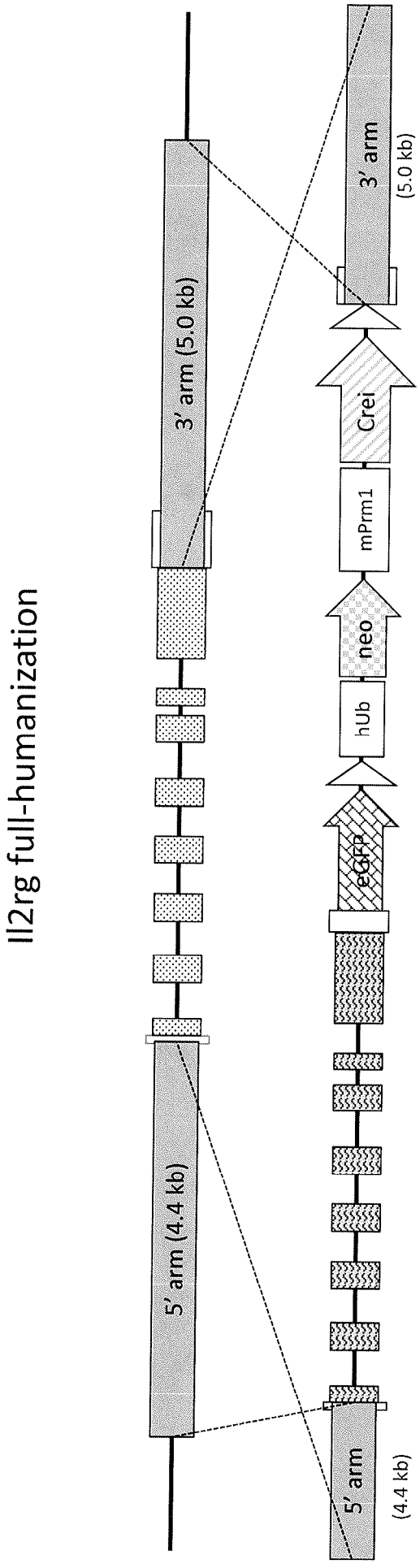

Il2rg full-humanization
3' arm (5.0 kb)
5' arm (4.4 kb)
3' arm
(5.0 kb)
Crei
mPrm1
neo
hUb
eGFP
5' arm
(4.4 kb)

FIG. 31

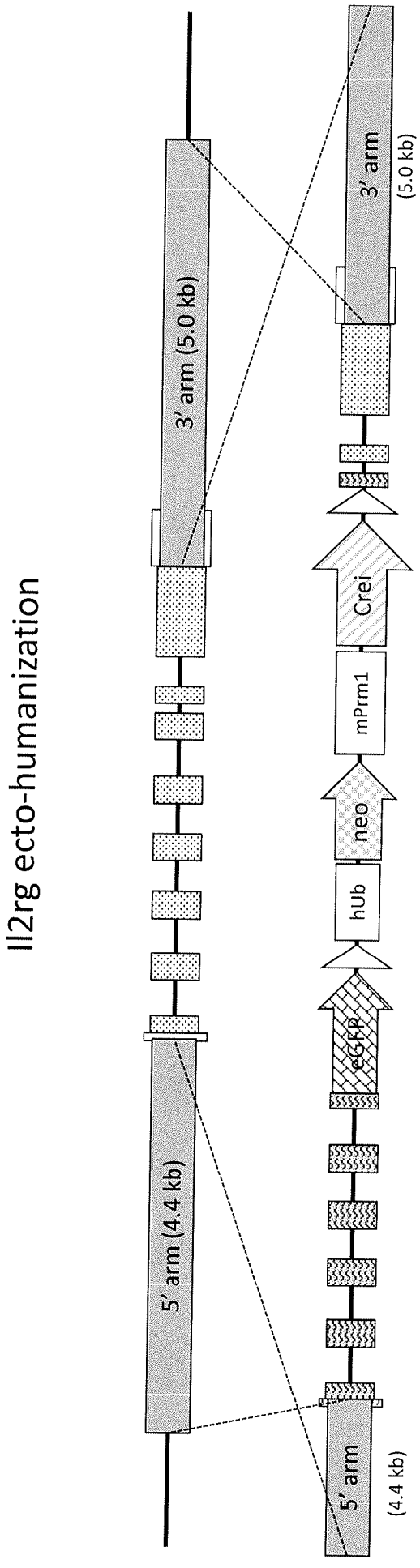

Il2rg ecto-humanization
3' arm (5.0 kb)
5' arm (4.4 kb)
3' arm
(5.0 kb)
Crei
mPrm1
neo
hUb
eGFP
5' arm
(4.4 kb)

FIG. 32

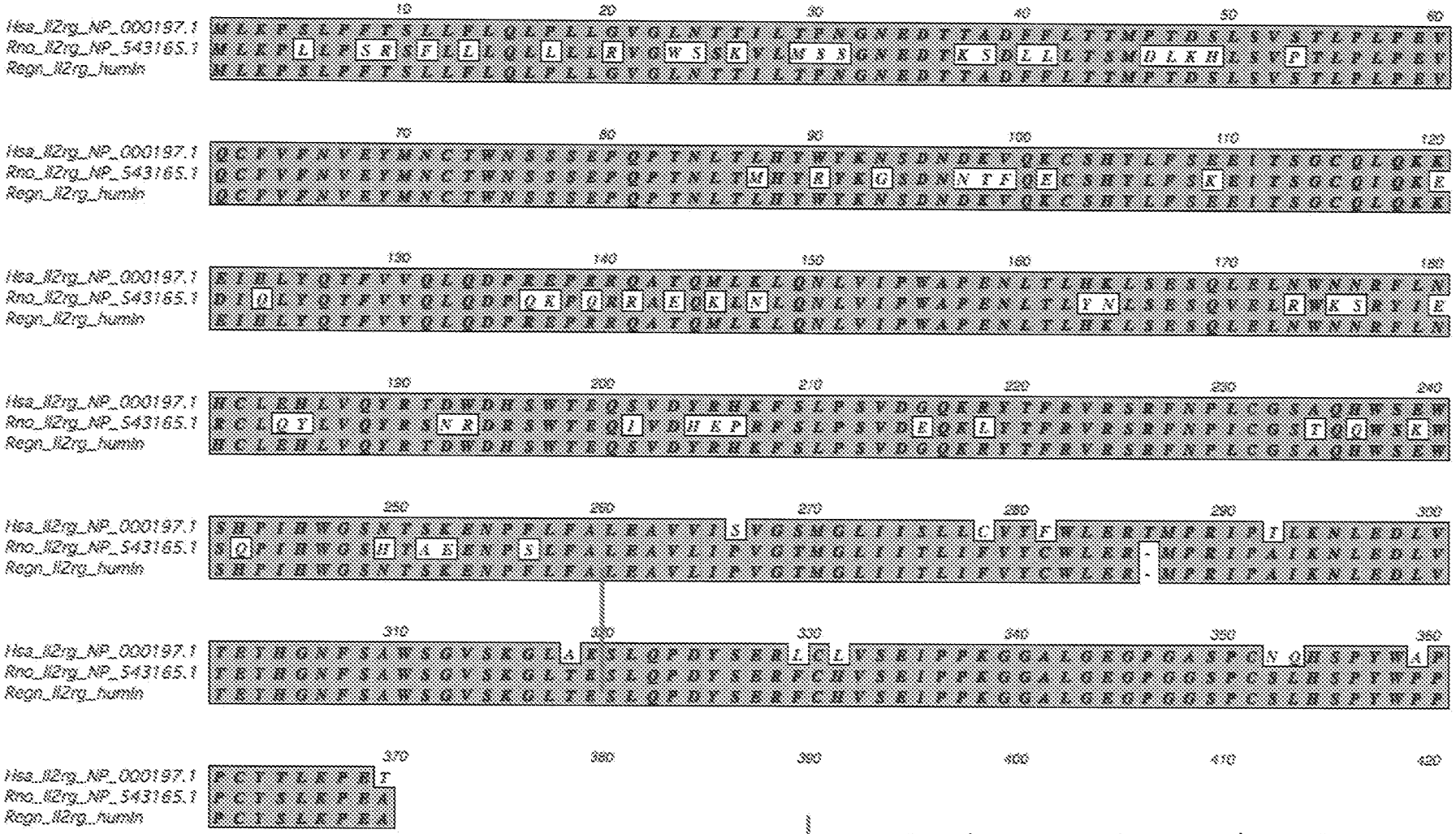

Hsa_Il2rg_NP_000197.1
Rno_Il2rg_NP_543165.1
Regn_Il2rg_humin
Junction between human/rat Il2rg protein

FIG. 33