

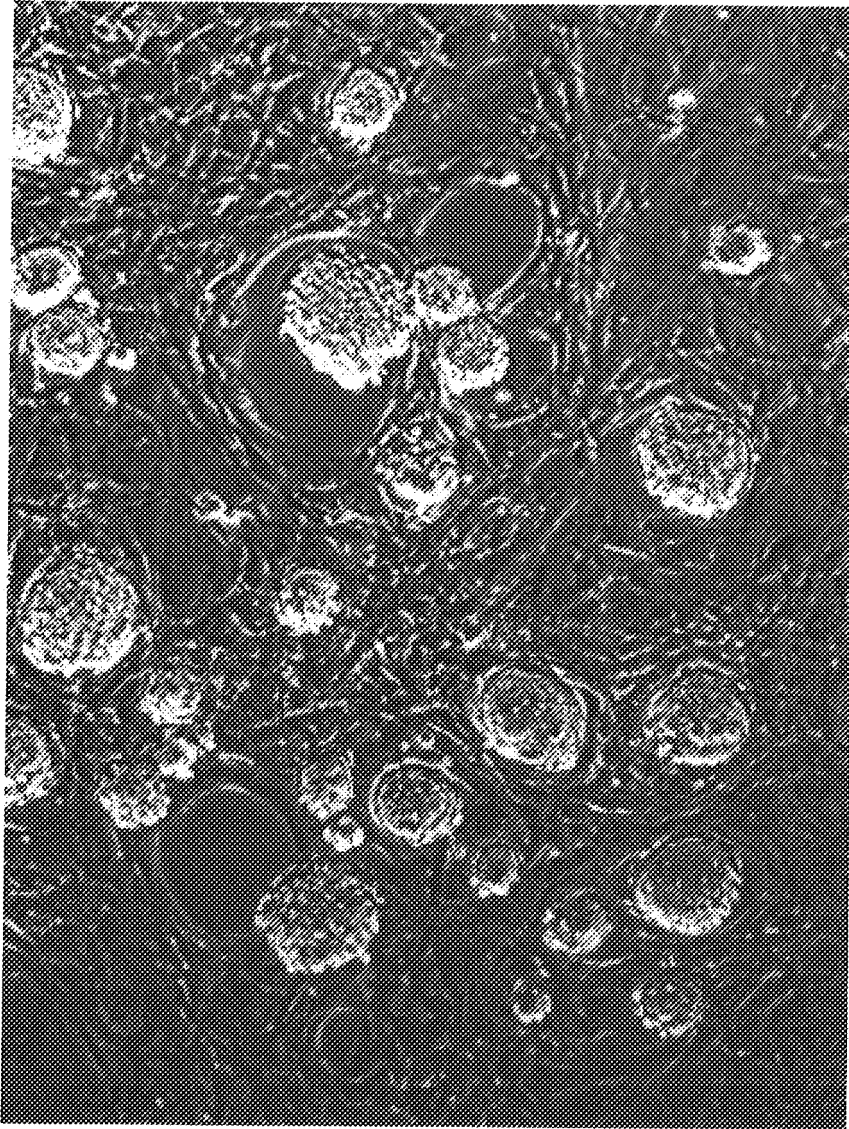


FIG. 1

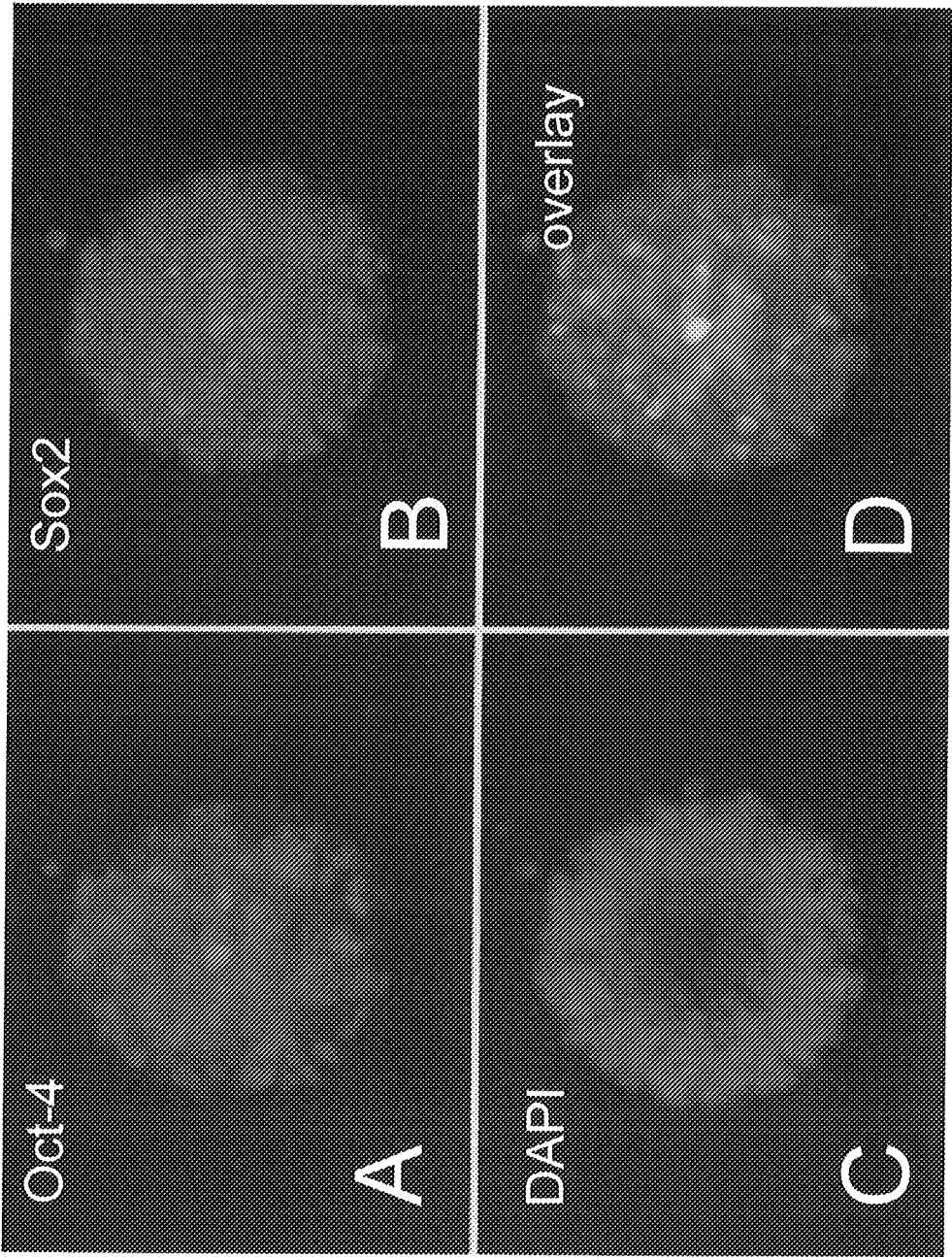


FIG. 2

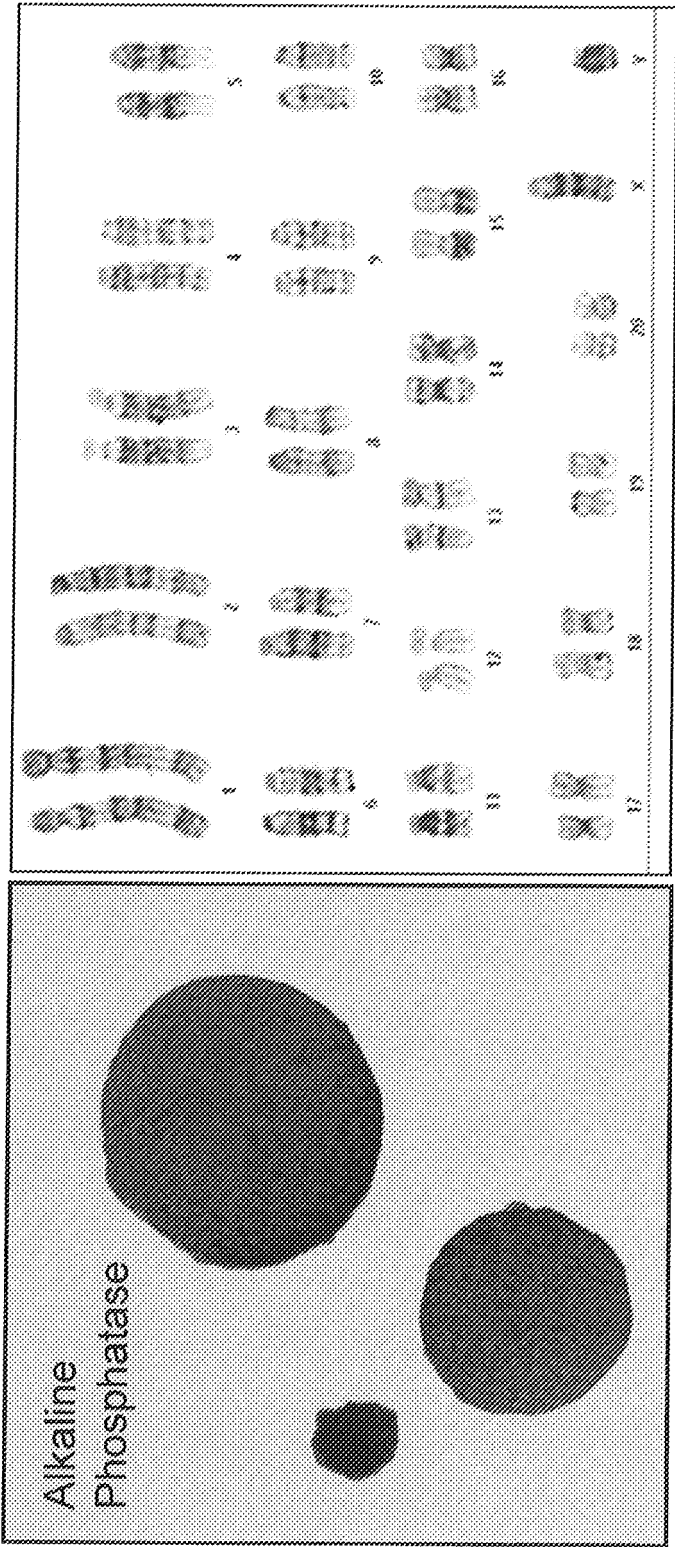


FIG. 3

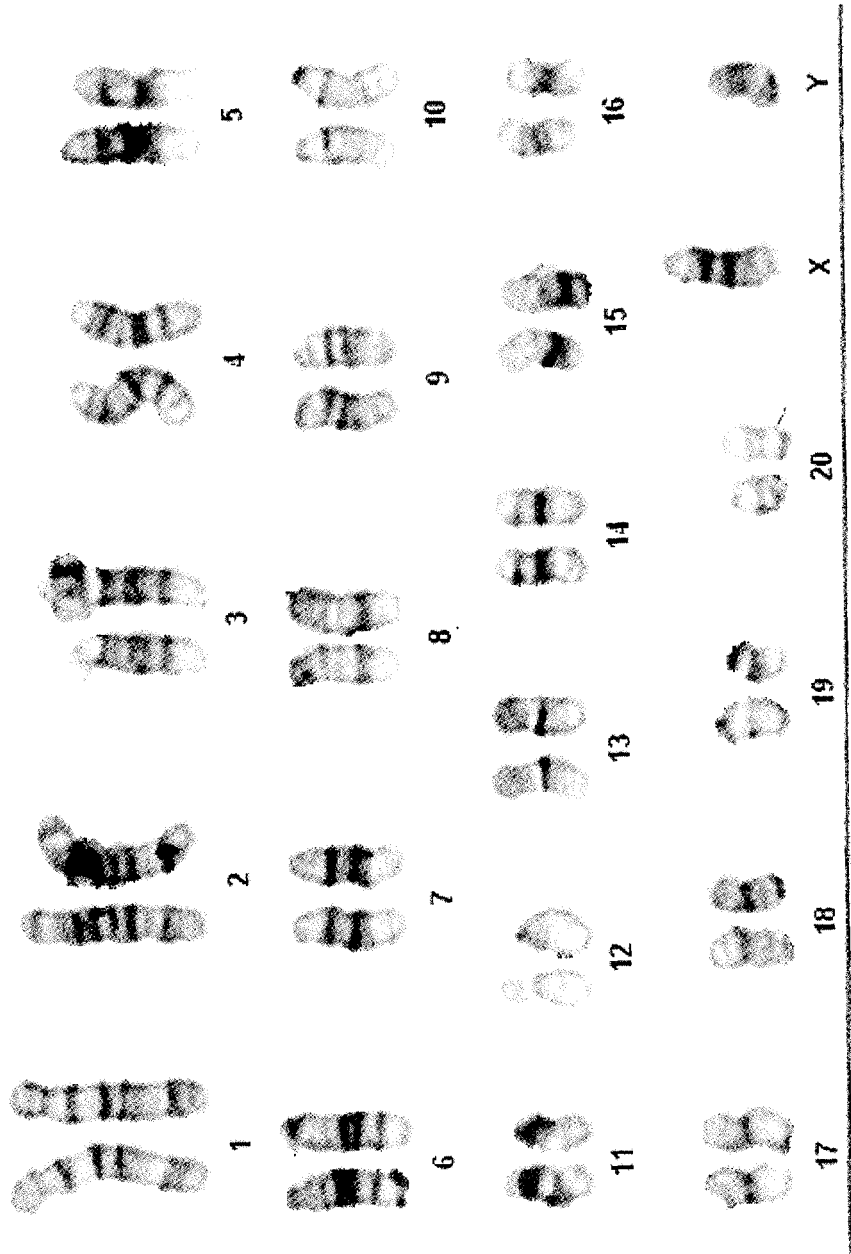
Cell Results: 42,XX



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

X, Y:
FIG. 4a

Cell Results: 42,XY

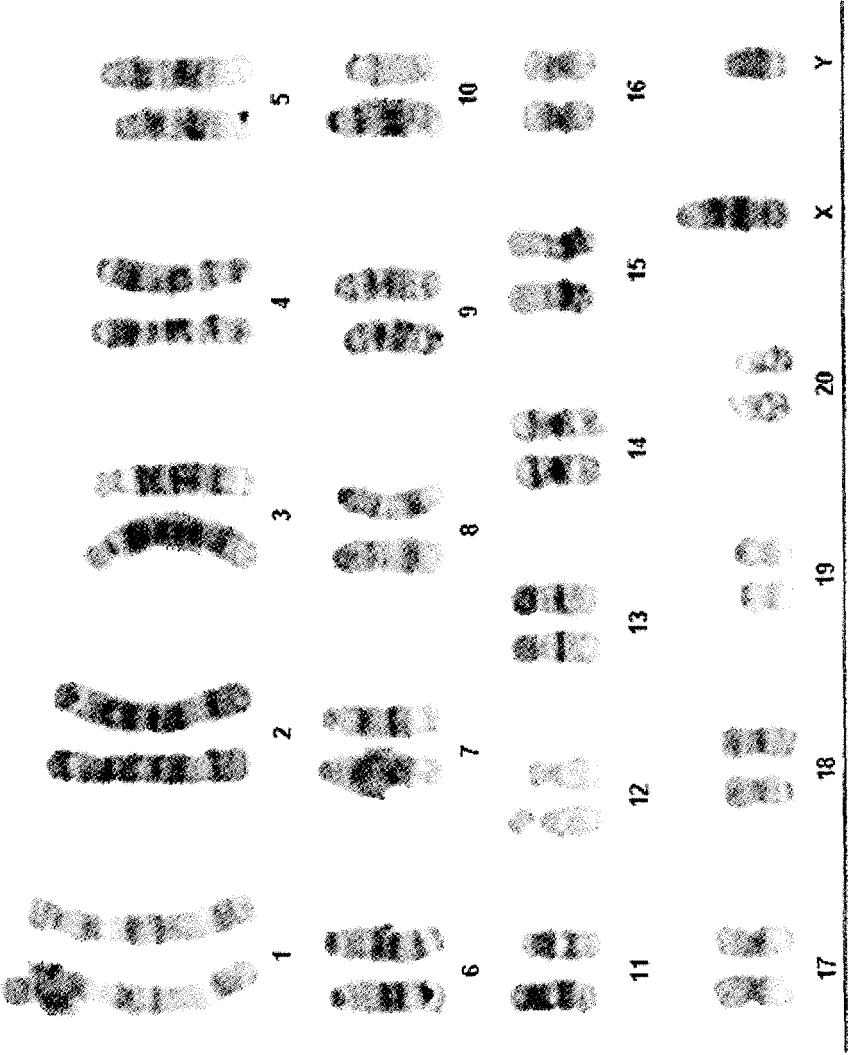


Label - Slide/Cell met-1 - S01-01

X, Y:

FIG. 4b

Cell Results: 42,XY



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

X, Y:

FIG. 5a

Cell Results: 42,XY

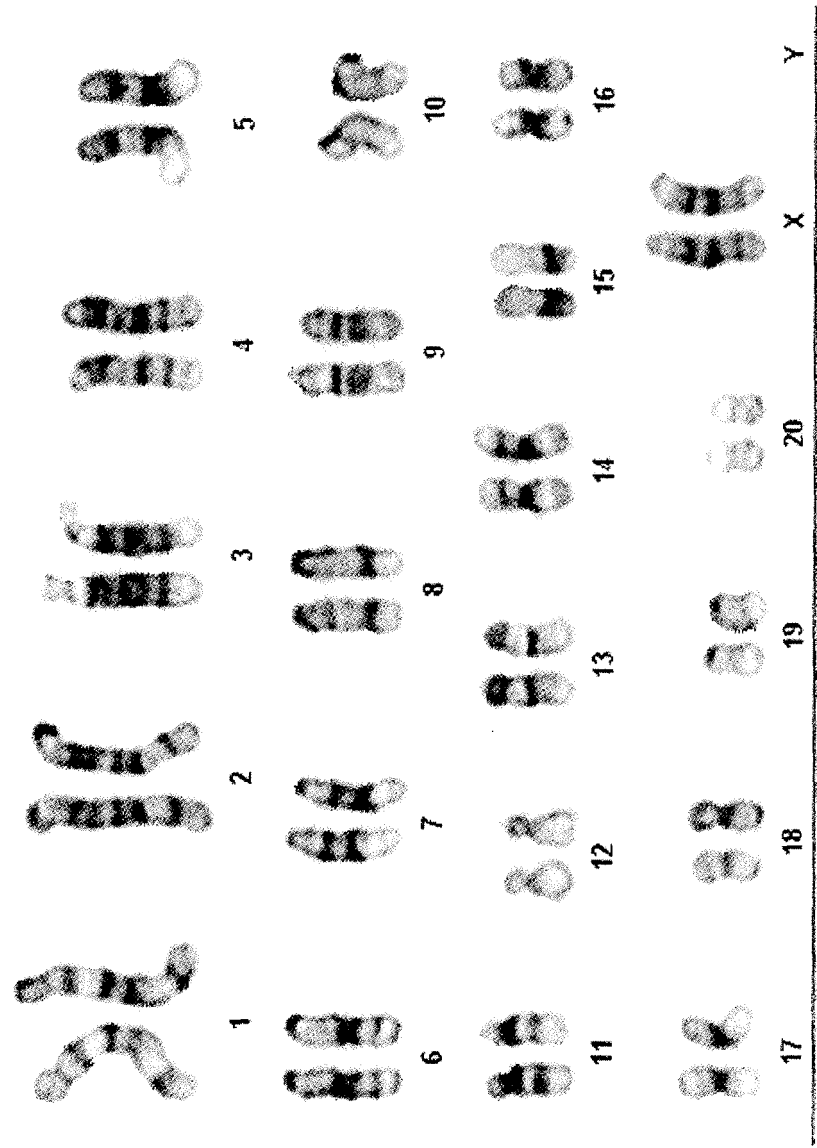


Label - Slide/Cell met-1 - S01-01

X, Y:

FIG. 5b

Cell Results: 42,XX



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

X, Y:

FIG. 6a

Figure 6b



X, Y:

FIG. 6b

Cell Results: 42,XX

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

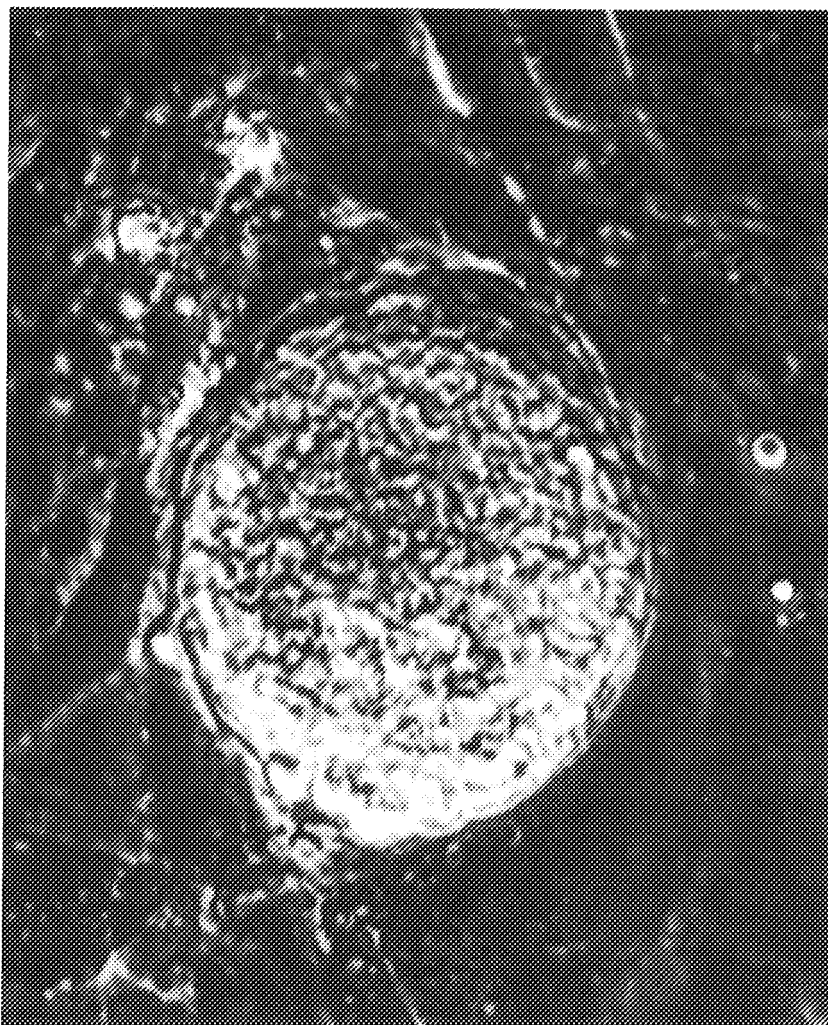


FIG. 7

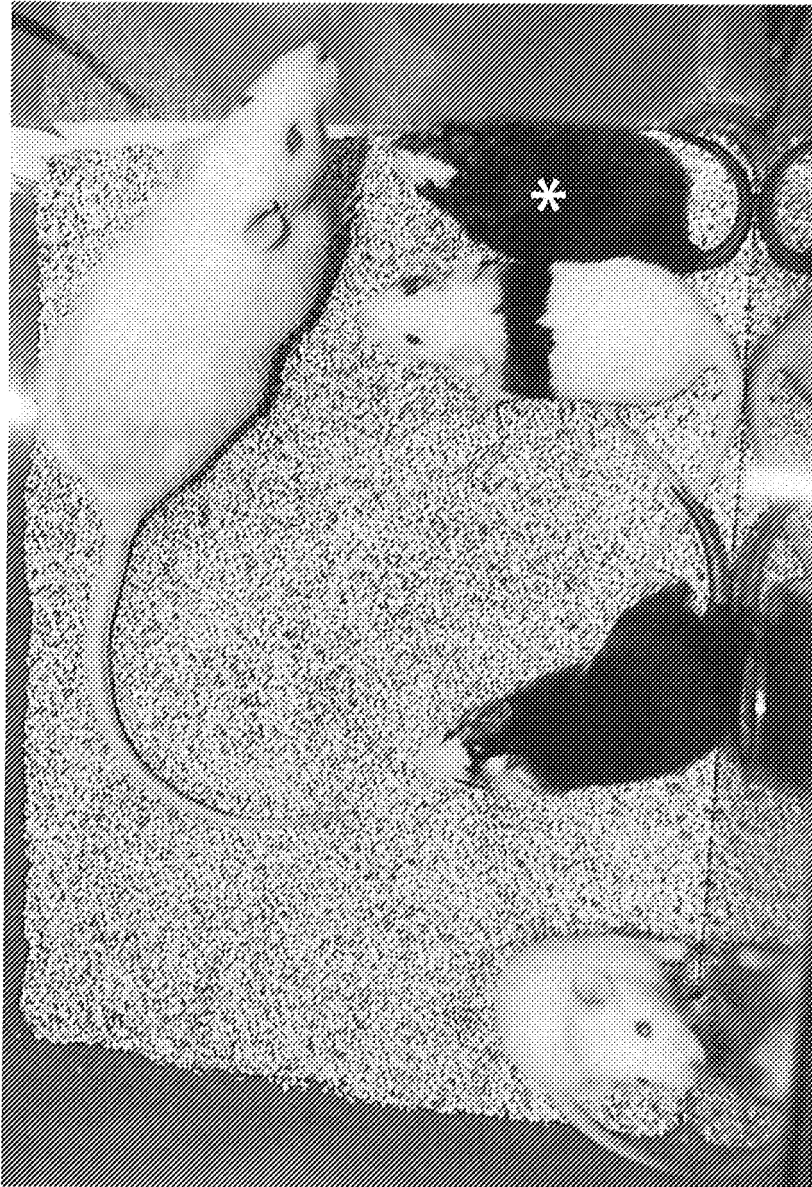


FIG. 8

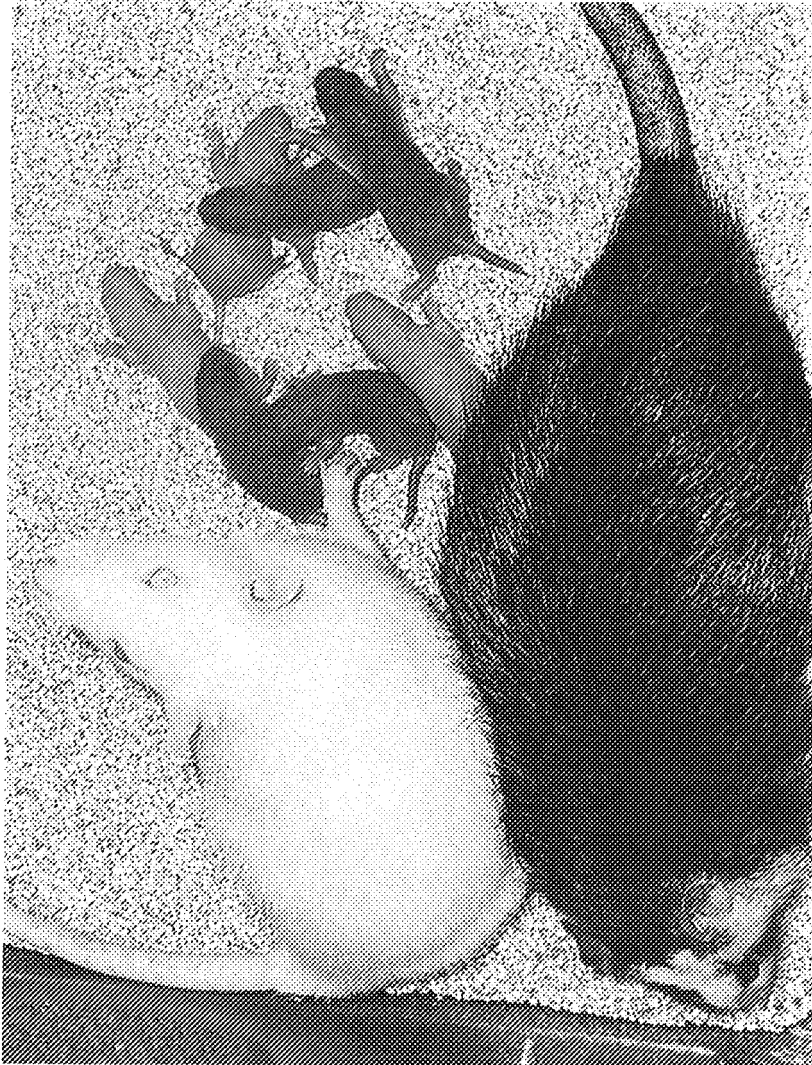


FIG. 9

Rat ApoE locus and Position of Zinc Finger Cutting Sites

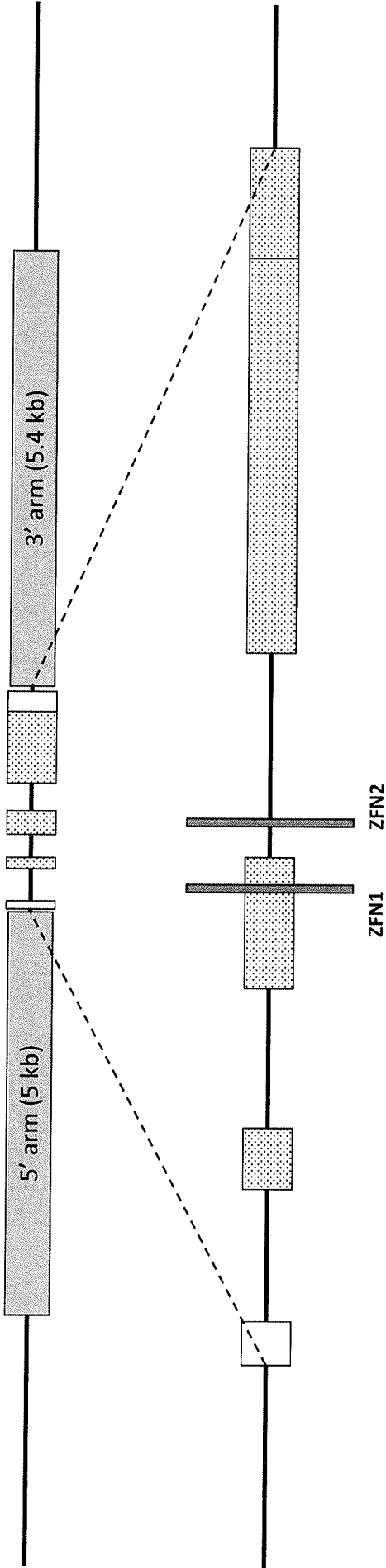


FIG. 10

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

1 copy of ZFN probe lost
2 copies of ZFN probe lost
2 copies of ZFN probe lost

FIG. 11

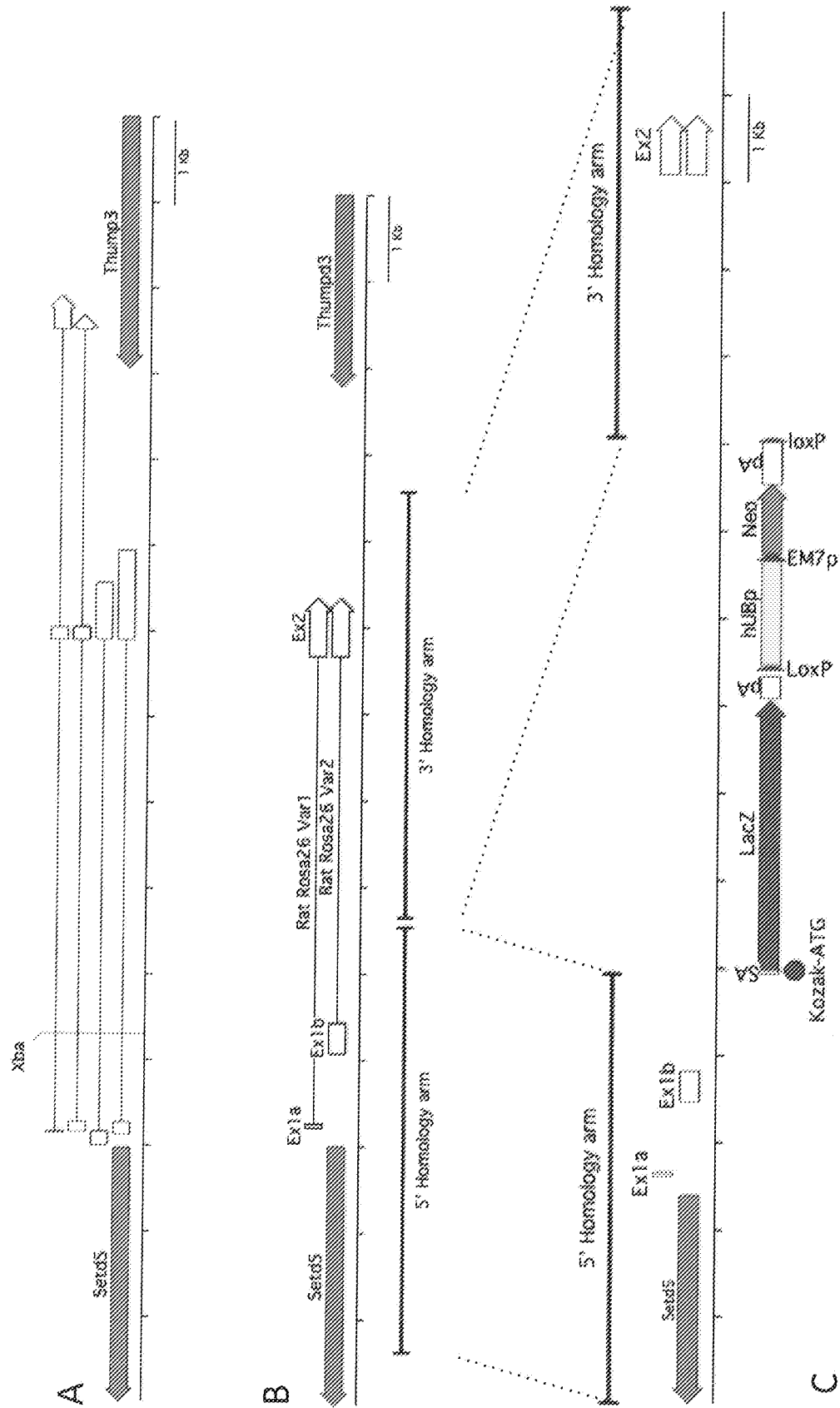


FIG. 12

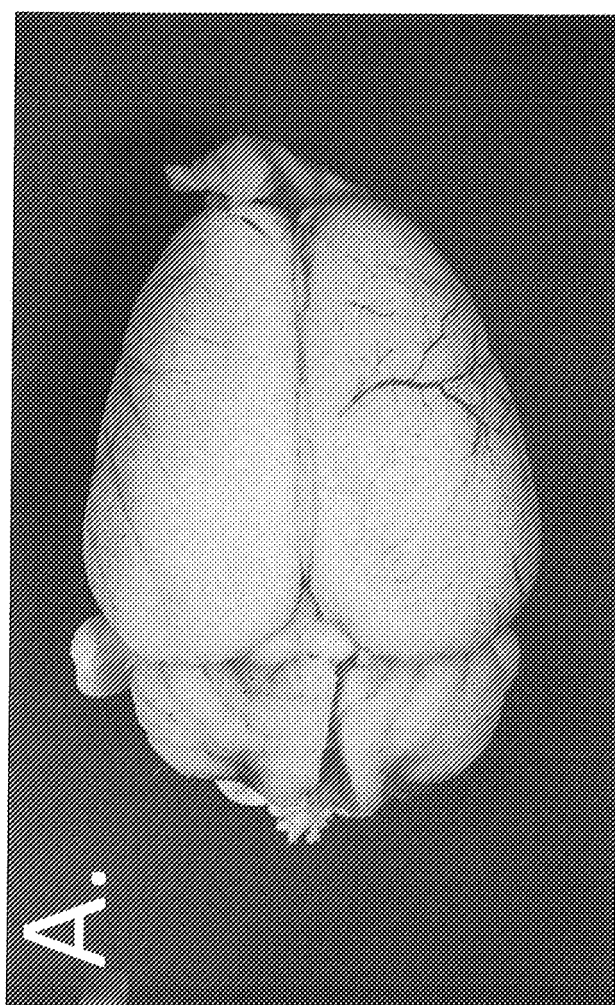


FIG. 13a

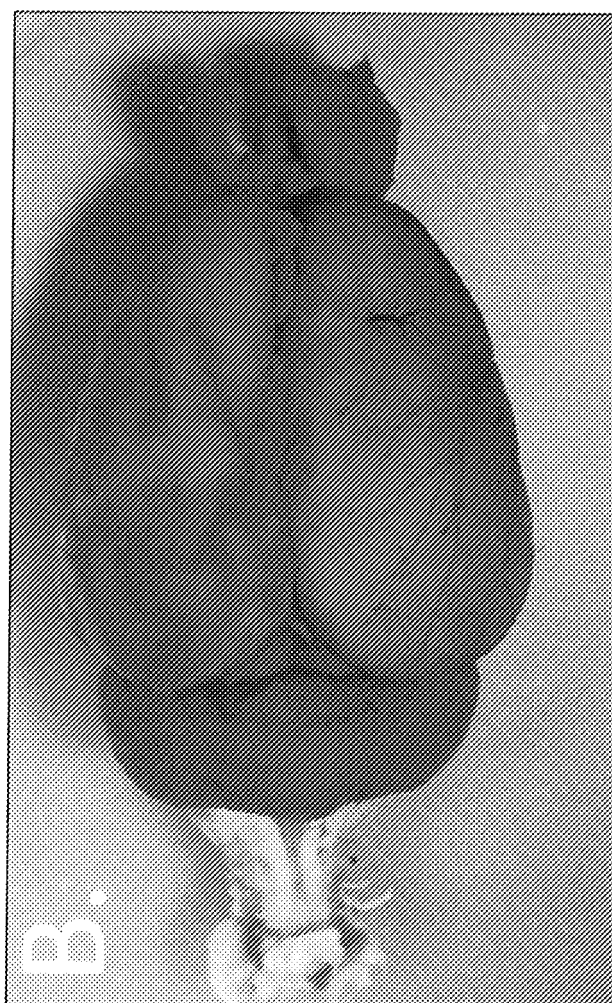


FIG. 13b

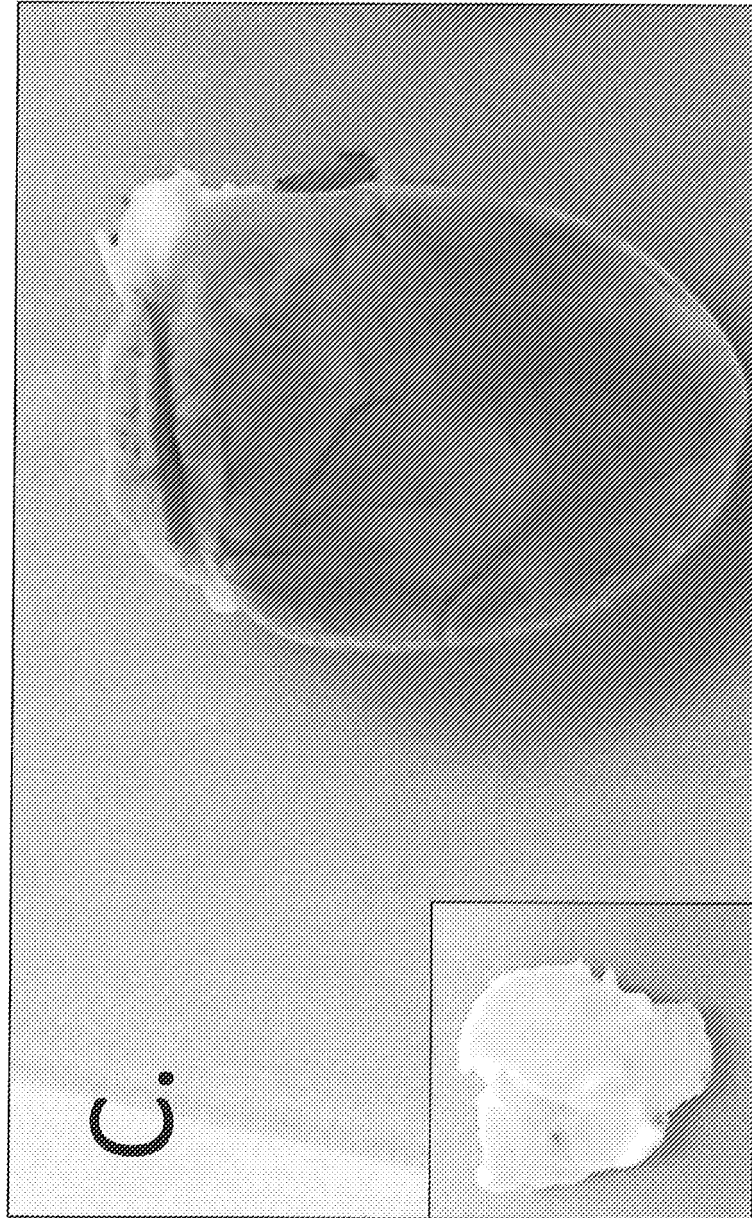


FIG. 13c

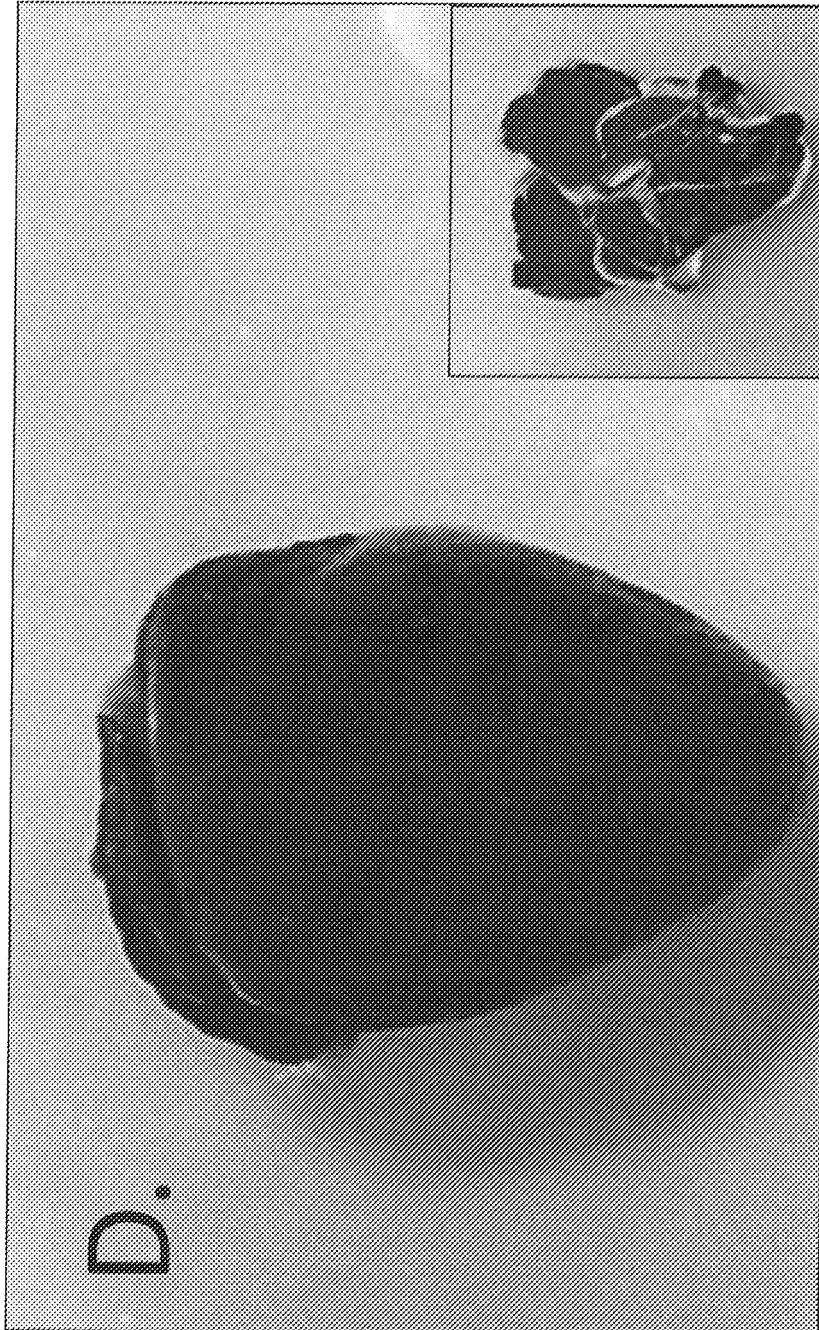


FIG. 13d

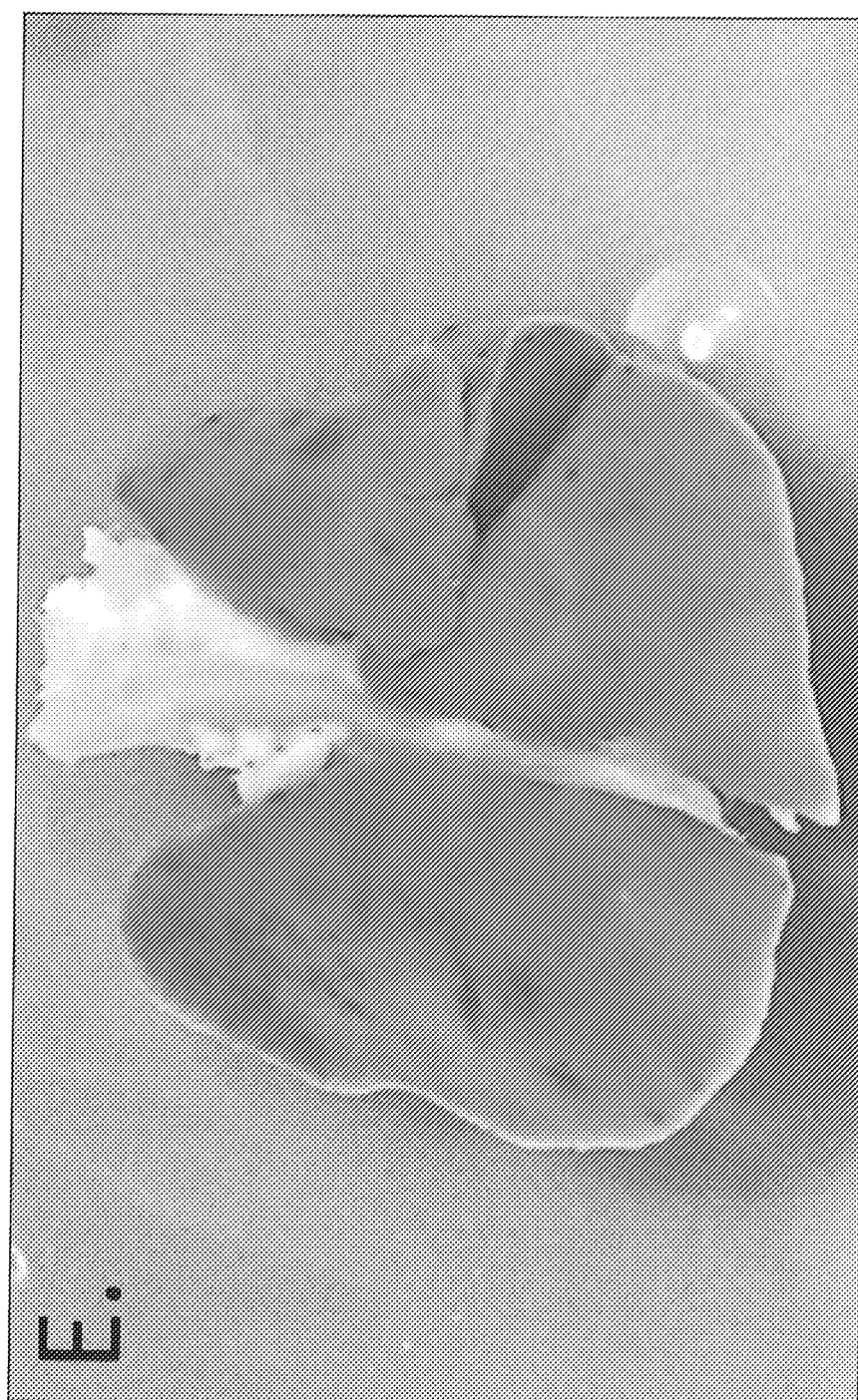


FIG. 13e

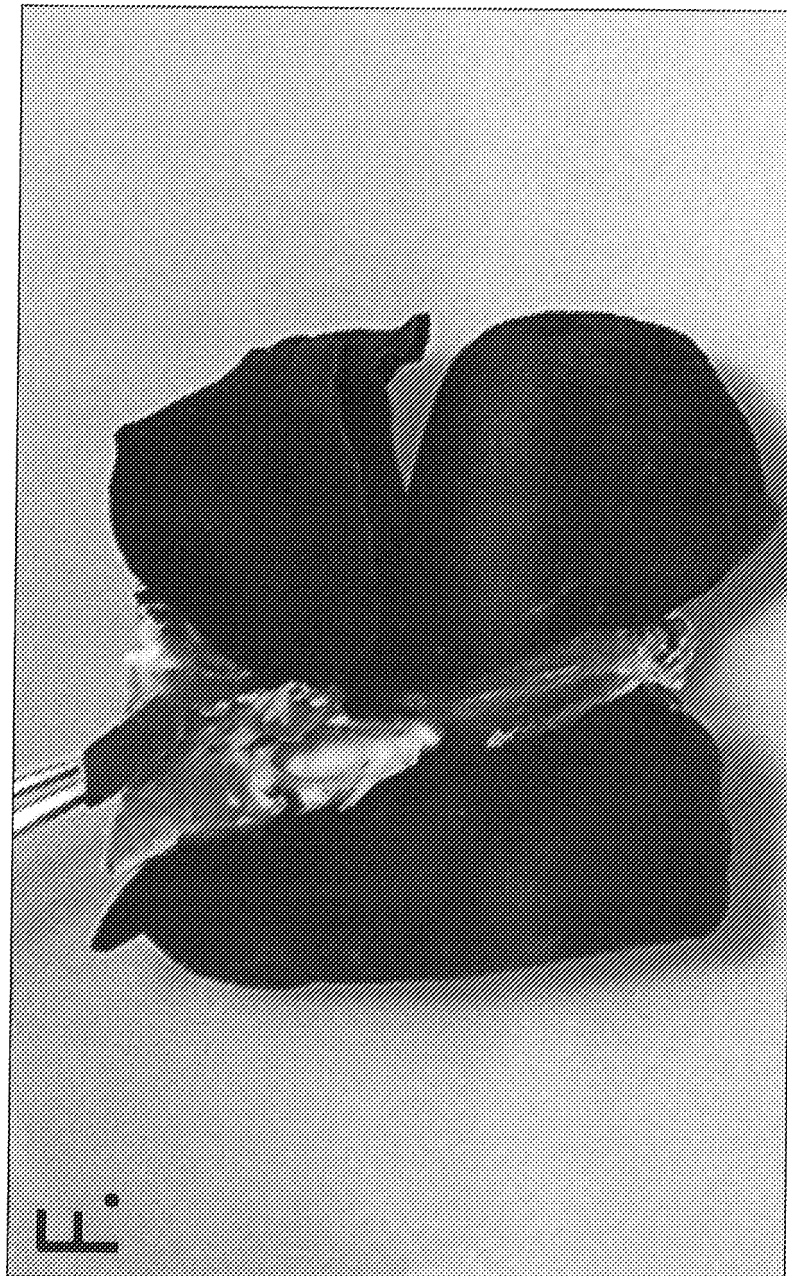


FIG. 13F

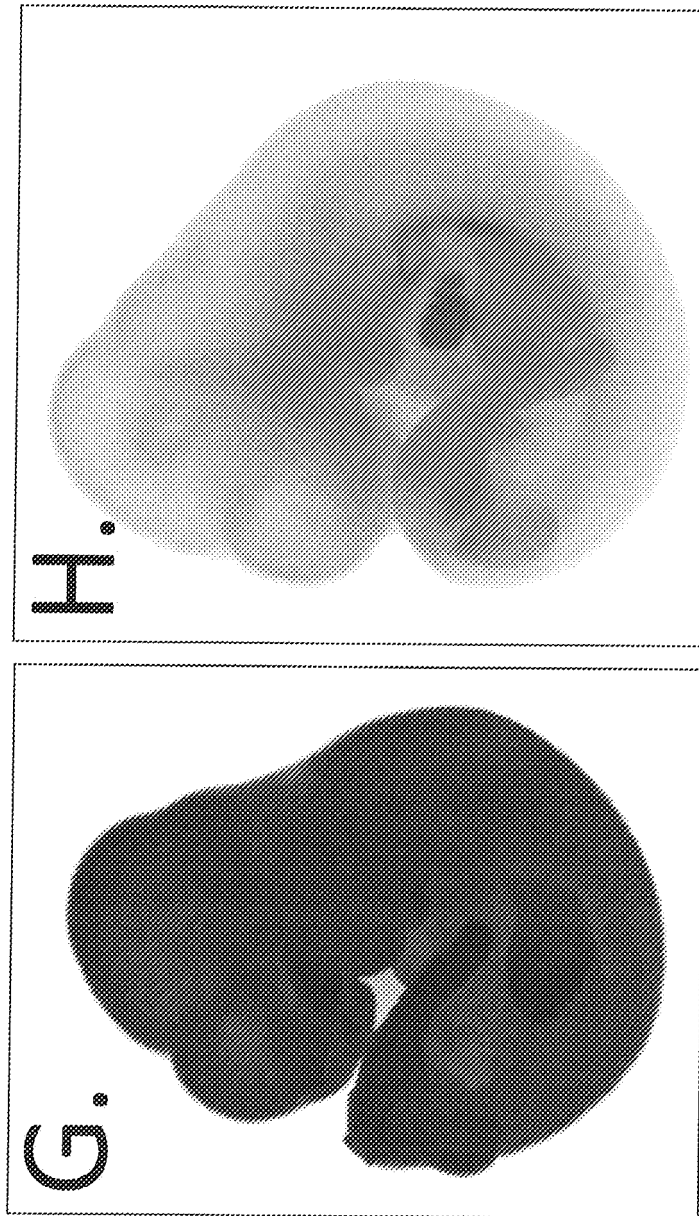


FIG. 13g, h

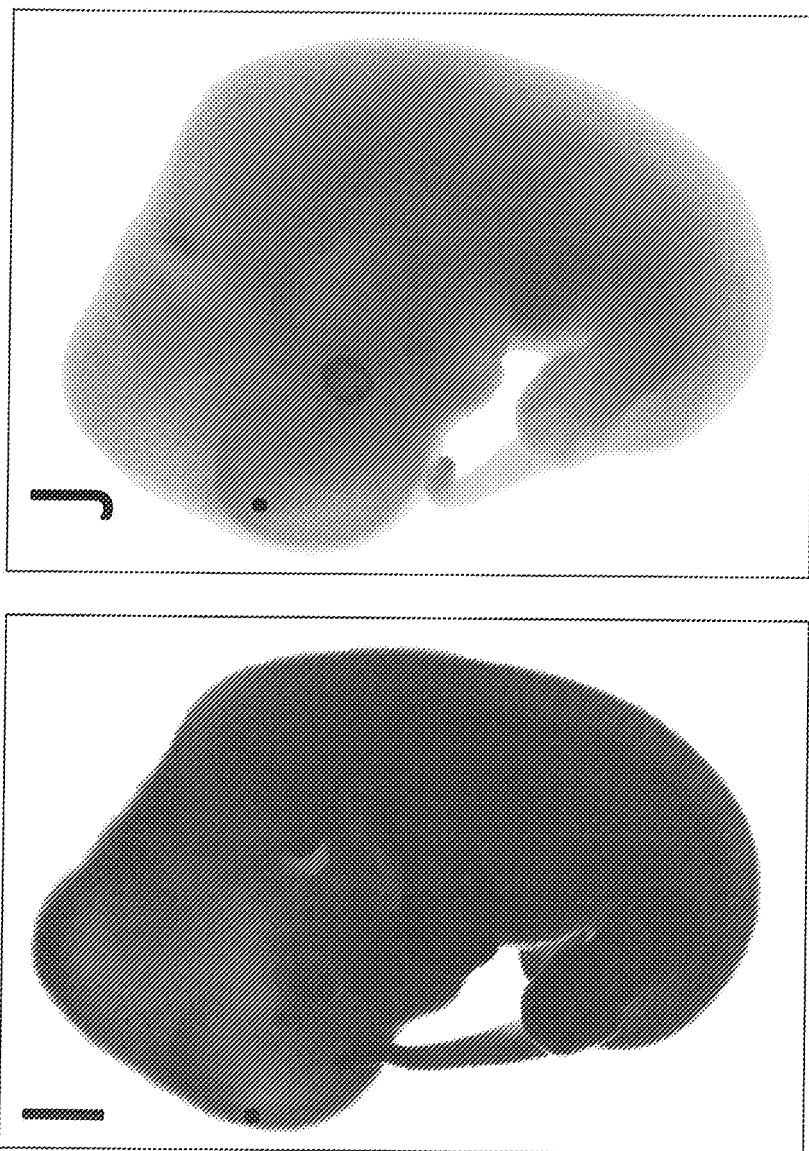
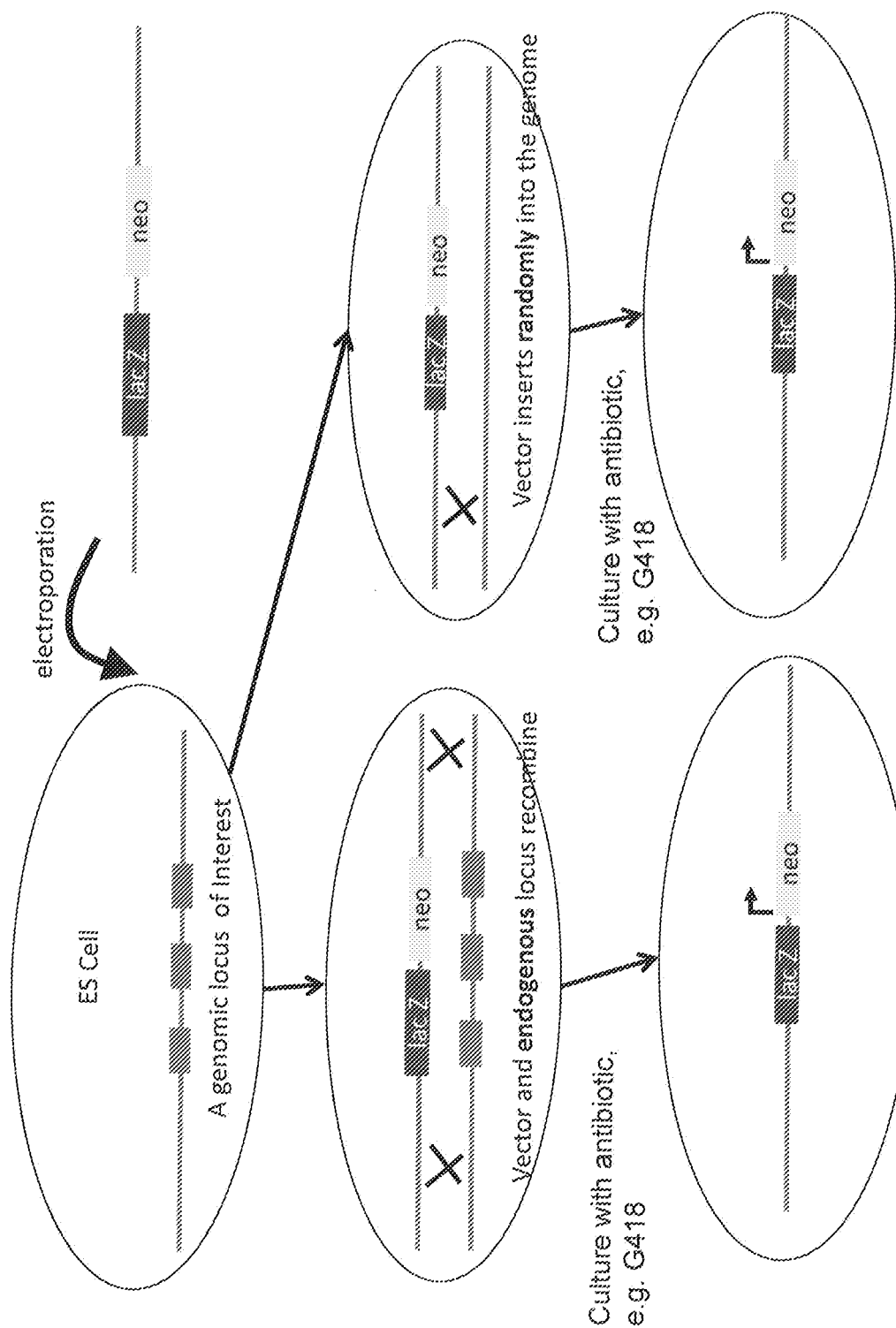


FIG. 13i,j



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

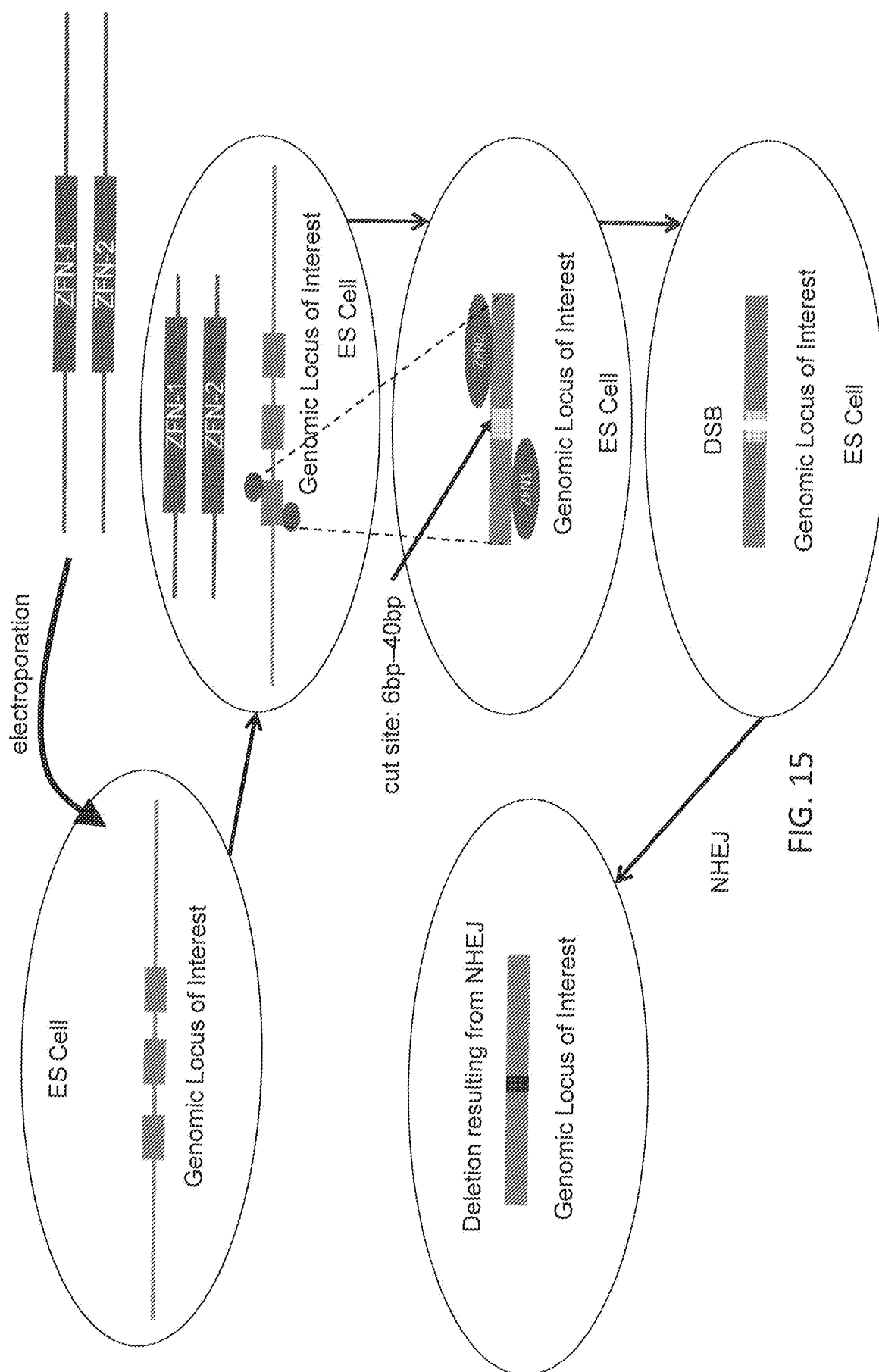
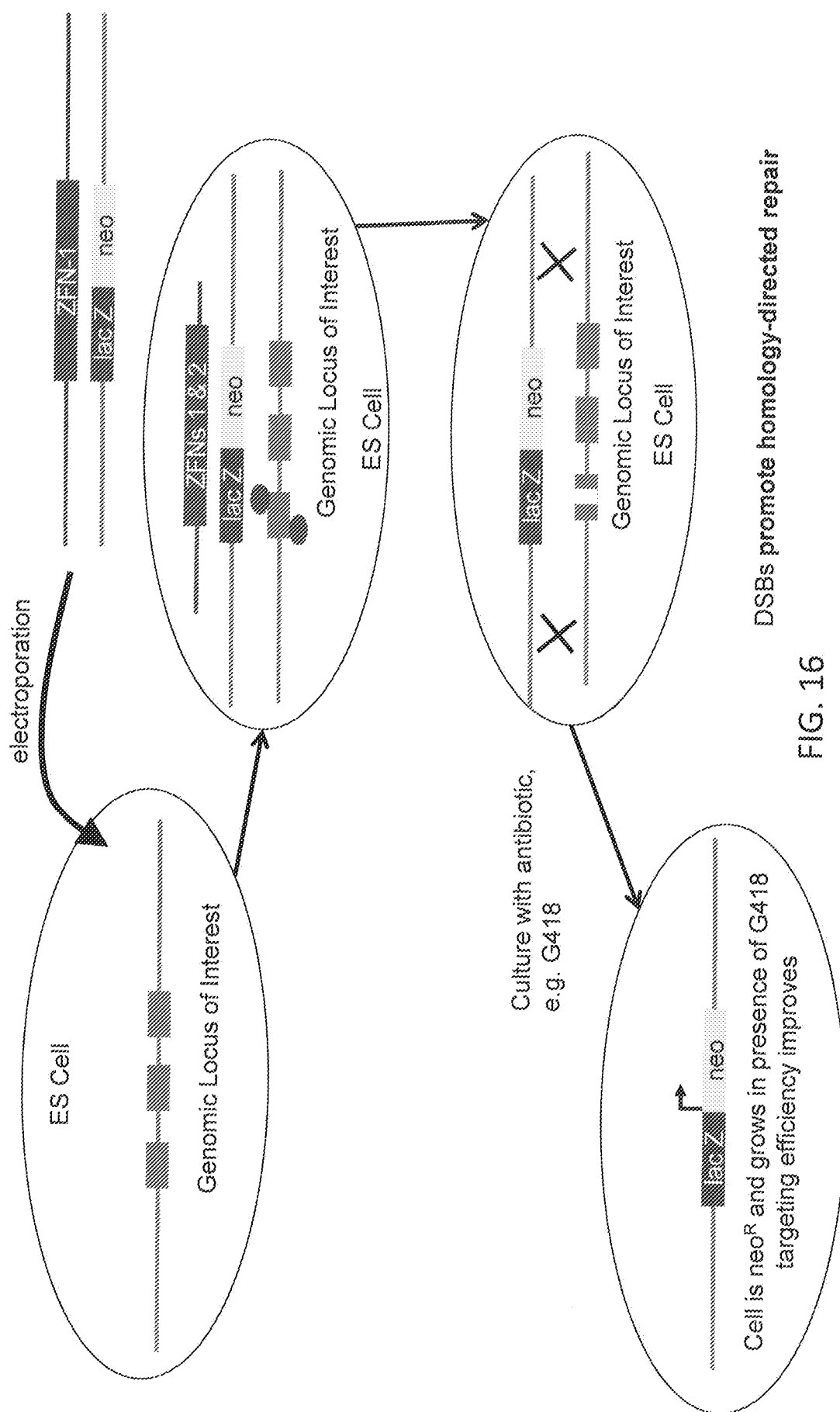


FIG. 15



DSBs promote homology-directed repair

FIG. 16

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



ApoE-ZFN1-AB5
chimeras

FIG. 17

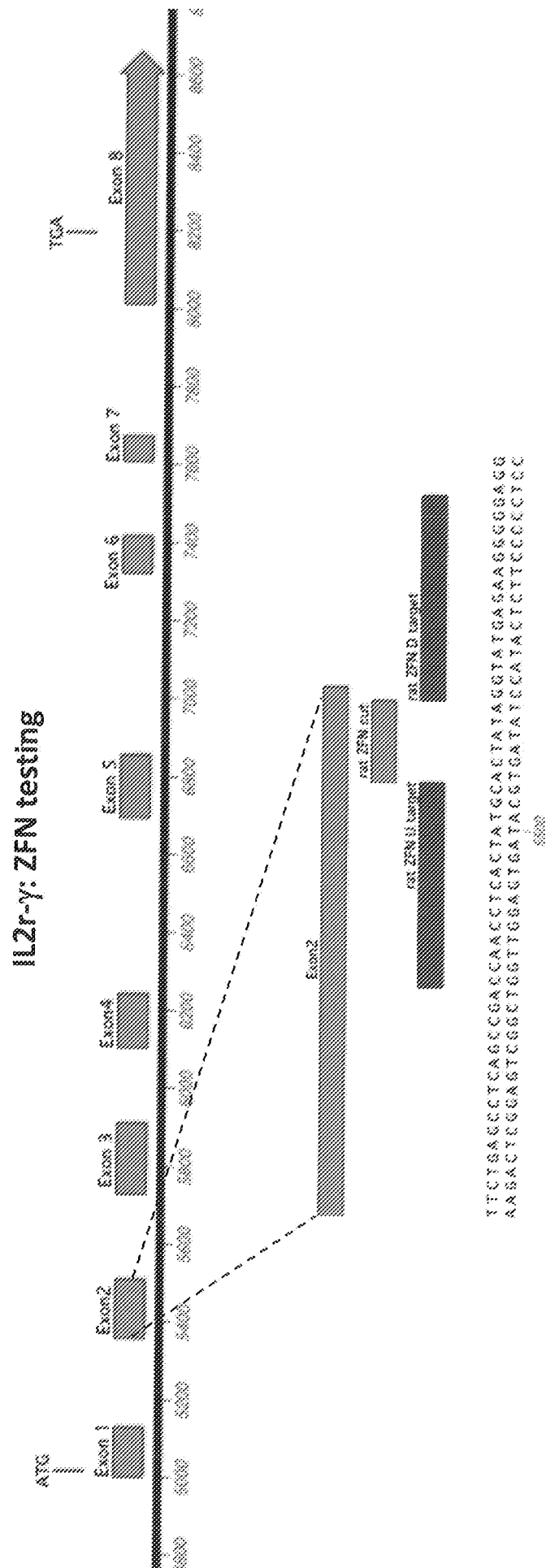
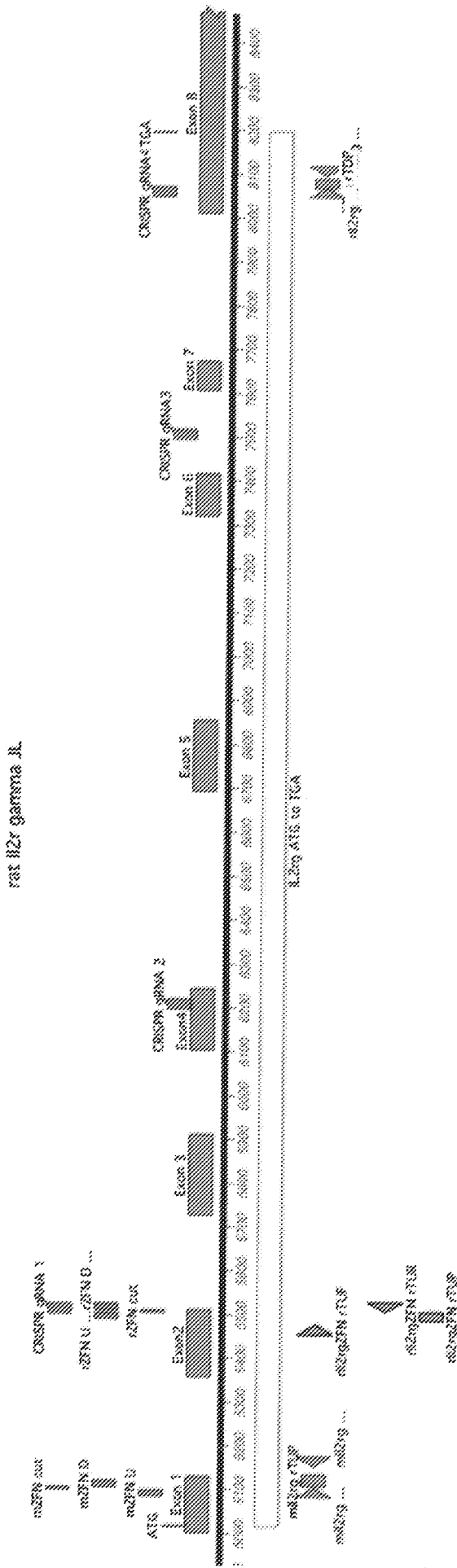


Plate	Colonies screened	Targeted	Efficiency
7/18: Vector alone	96	4	4.2%
7/18: Vector + ZFN	96	3	3.1%

ॐ
ॐ
ॐ
ॐ



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

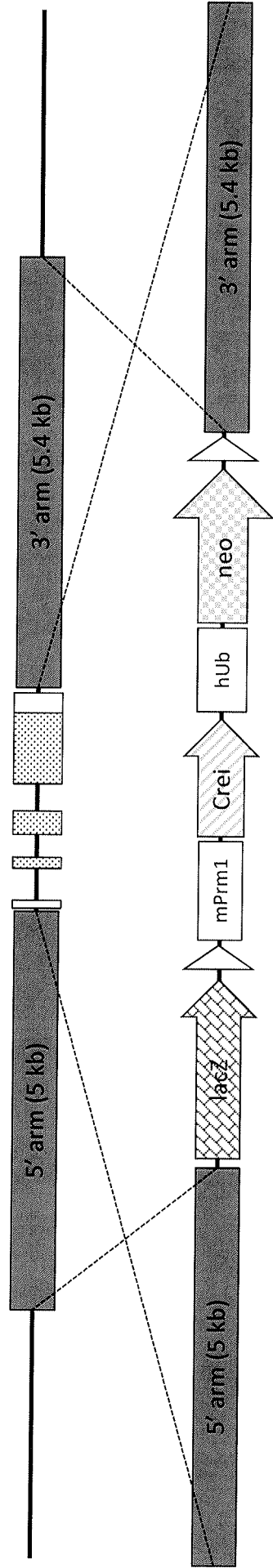
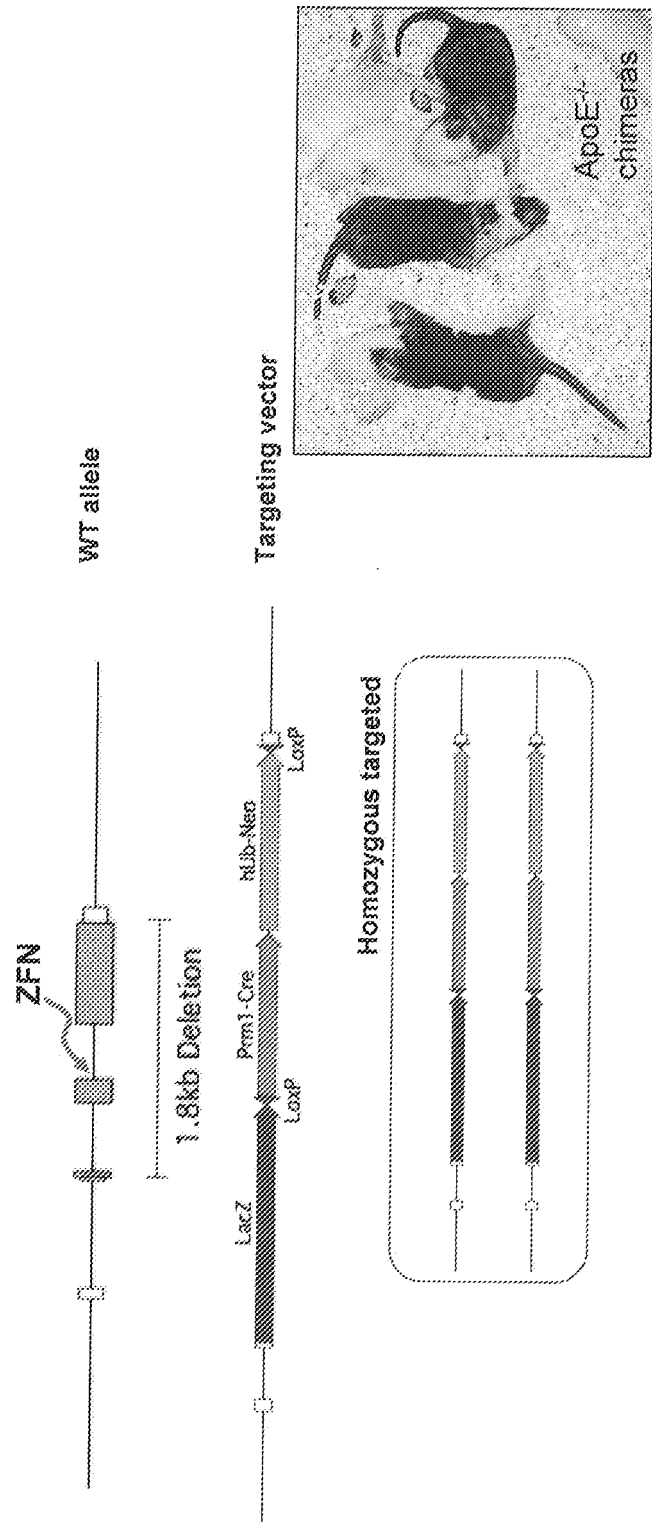


FIG. 20



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

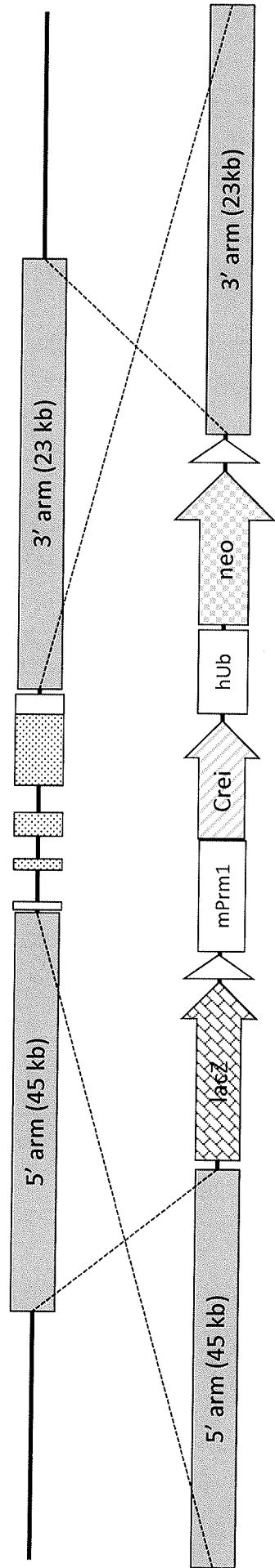


FIG. 22

Rat ApoE locus and Position of Zinc Finger Cutting Sites

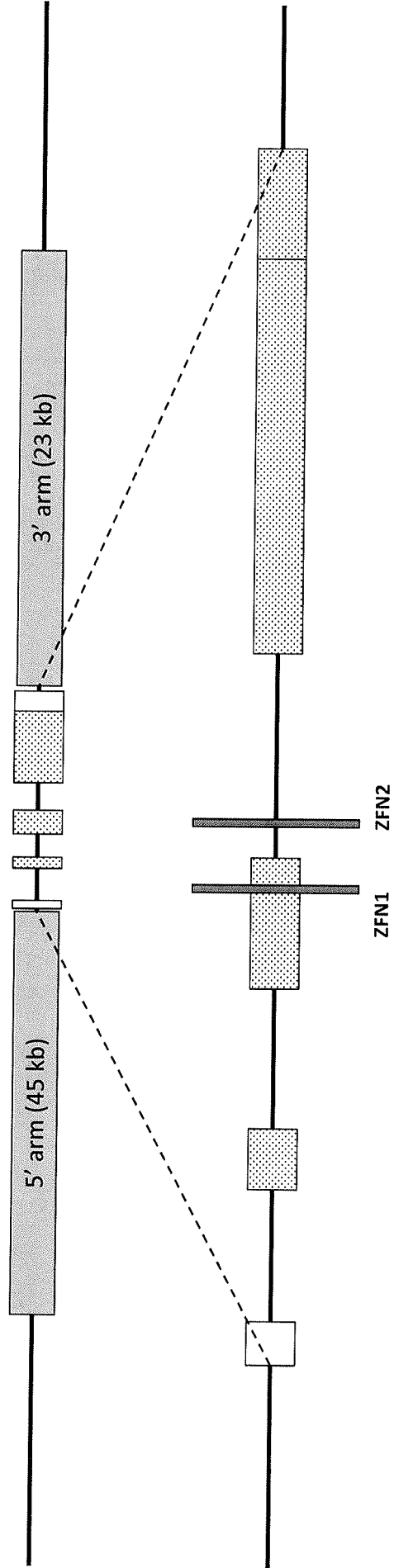


FIG. 23

Il2rg deletion

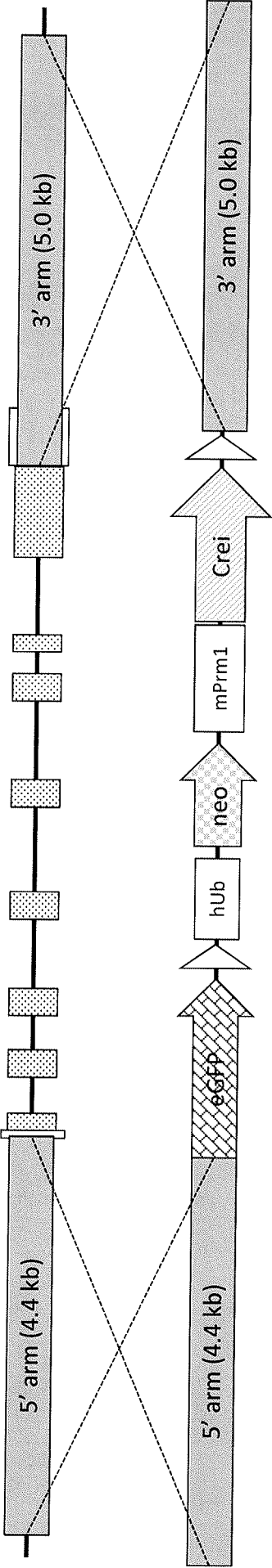


FIG. 24

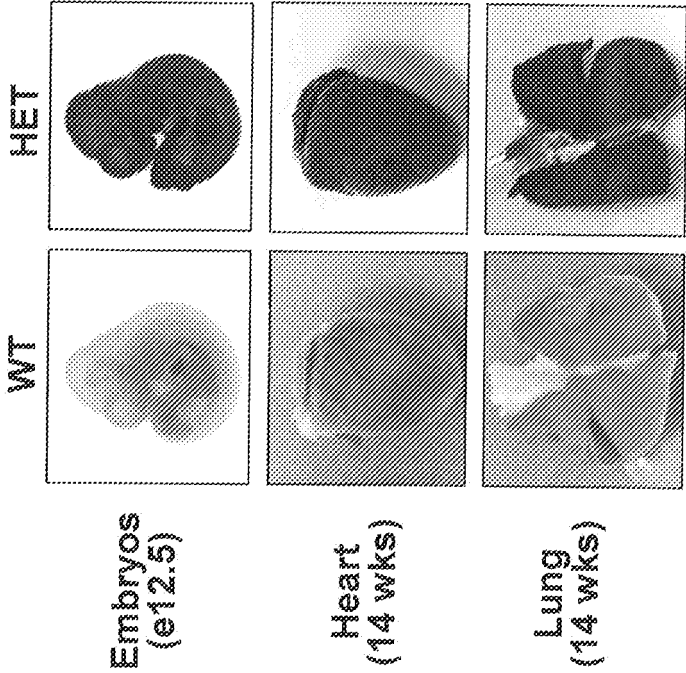
Germline Transmission Confirmed

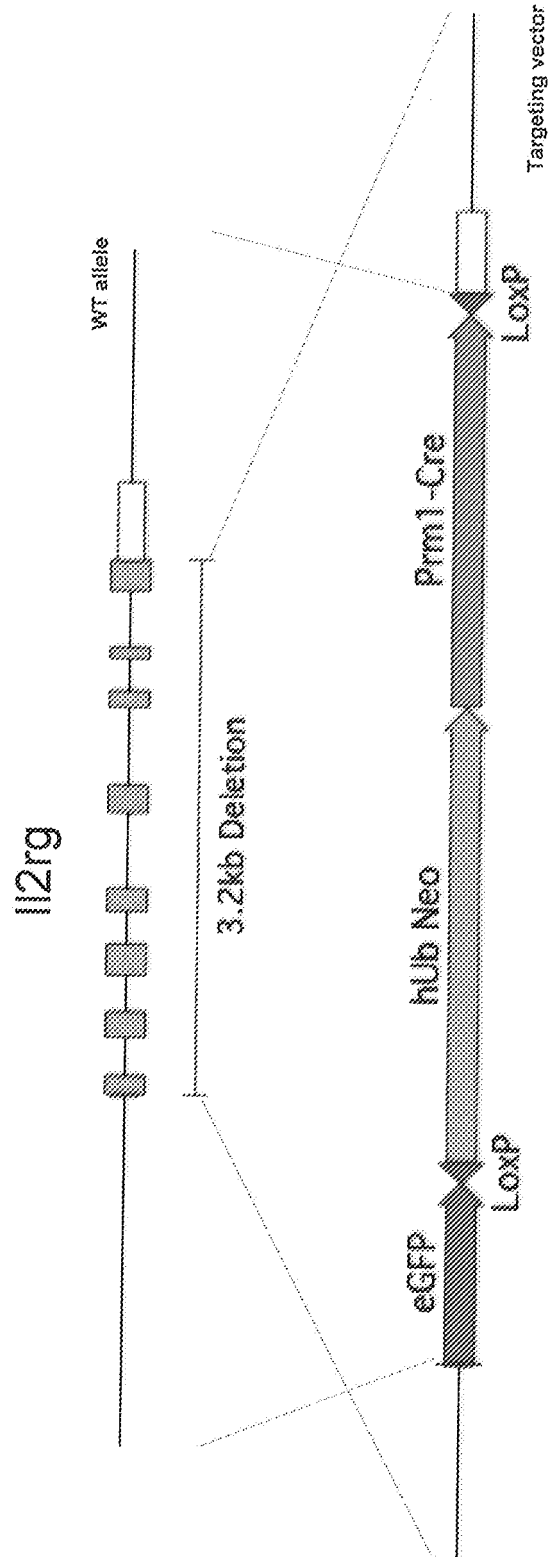
Line	Karyo- type	Chimera s bred	Germline transmitters	rESC- derived pups	GLT (%)
ACI/G1	XY	5	3 (60%)	11/103	11%
Dark Agouti 2B	XY	5	4 (80%)	11/129	9%
Dark Agouti 2C	XX	3	2 (66%)	7/45	16%

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

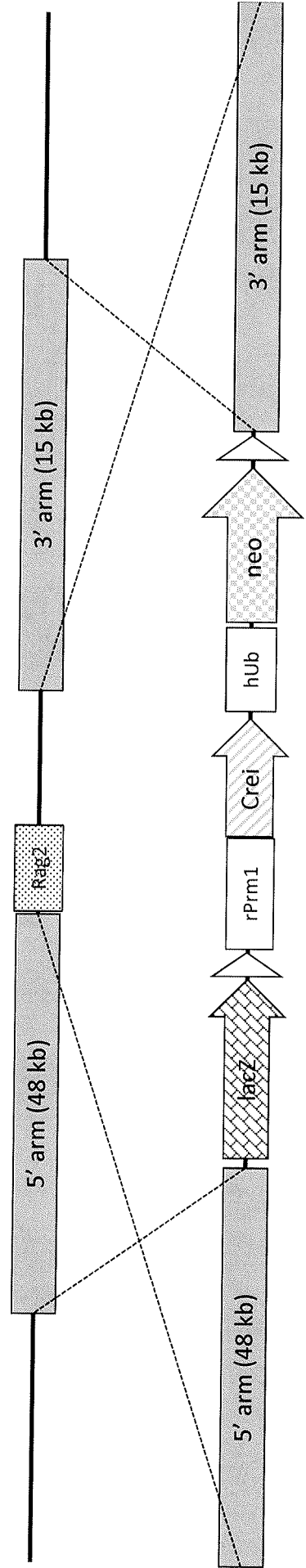


FIG. 27

Rag2/Rag1 targeting

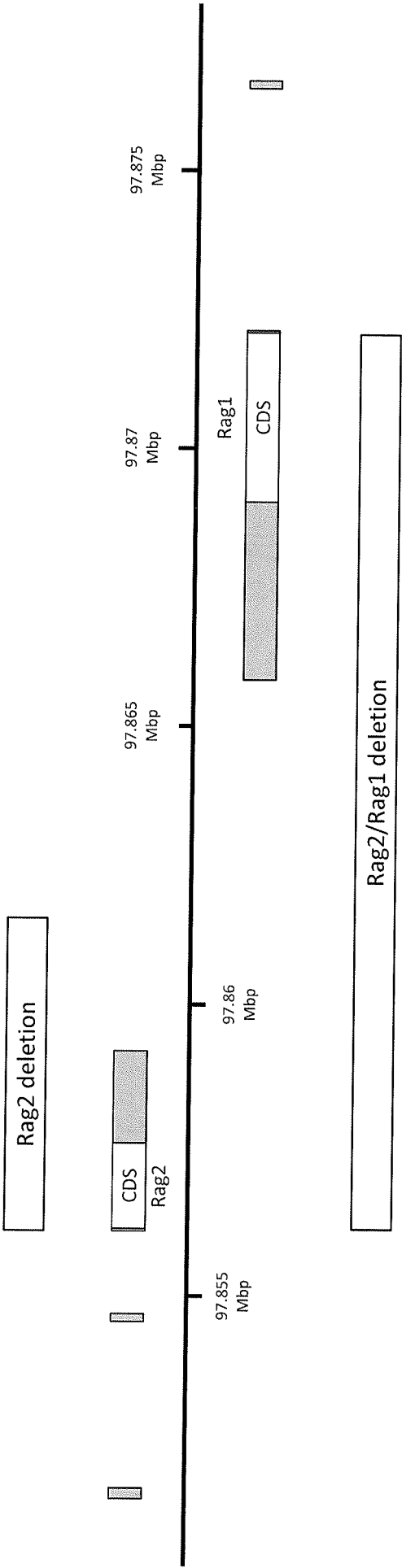


FIG. 28

Rag2/Rag1 targeting

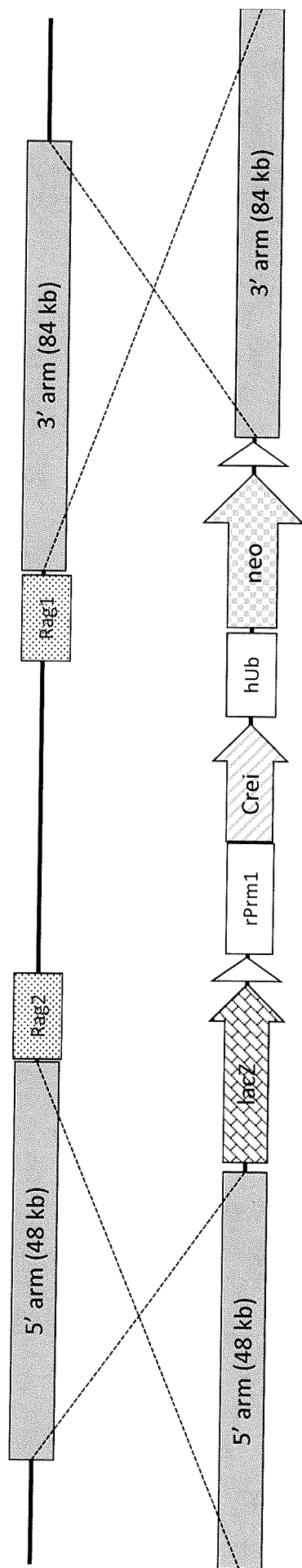


FIG. 29

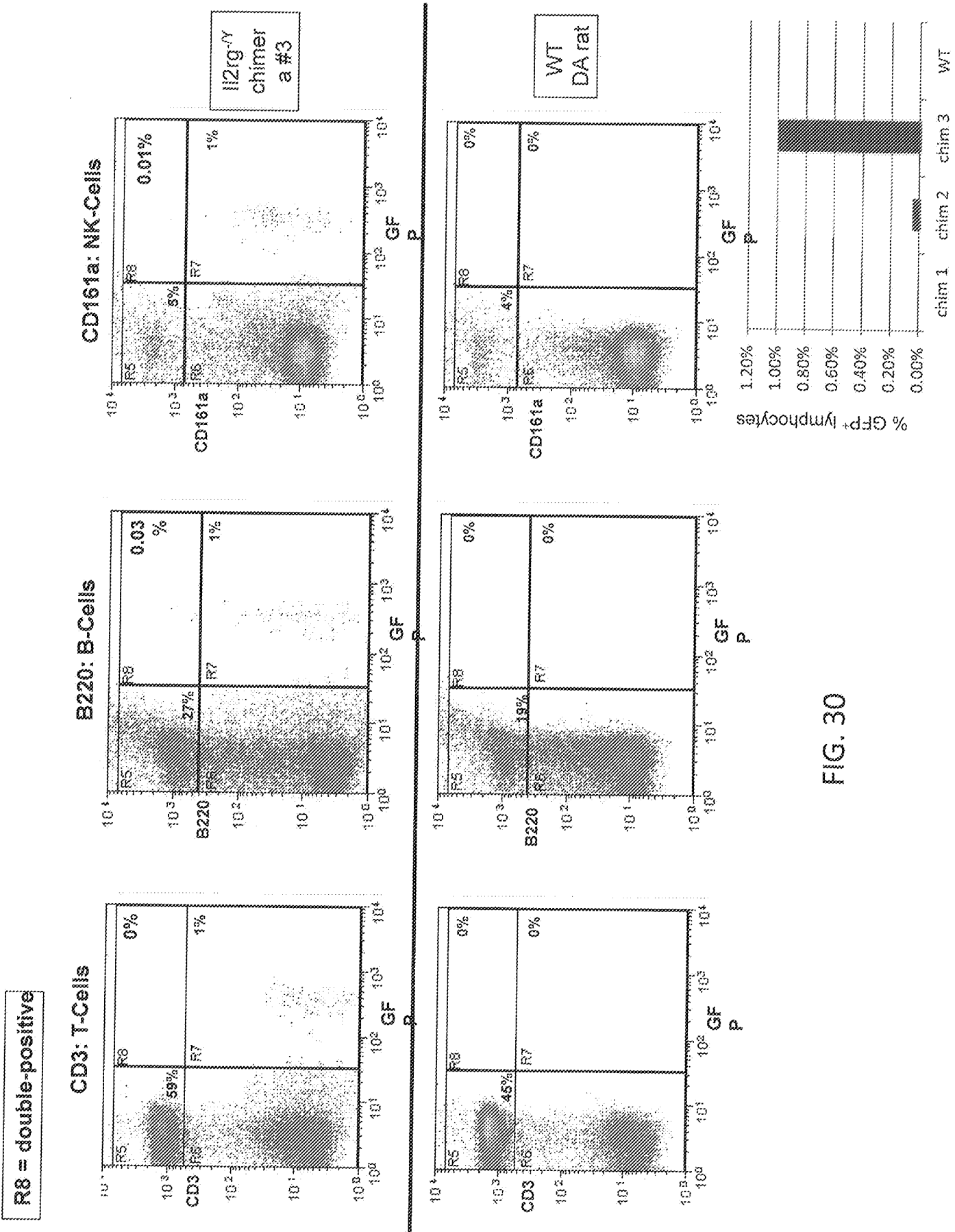


FIG. 30

IL2rg full-humanization

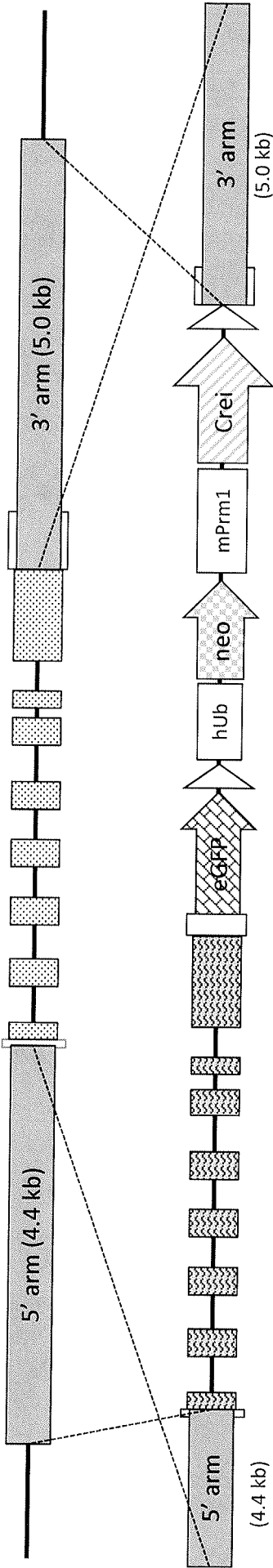


FIG. 31

Il2rg ecto-humanization

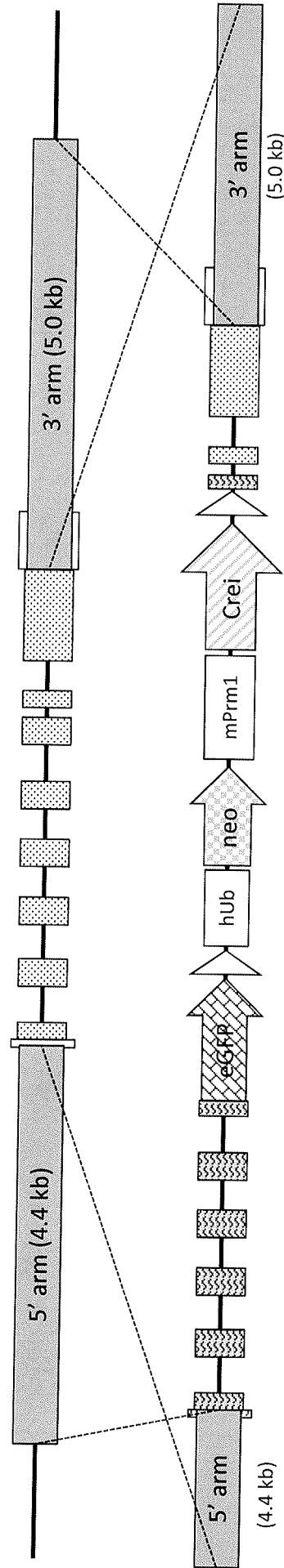


FIG. 32

