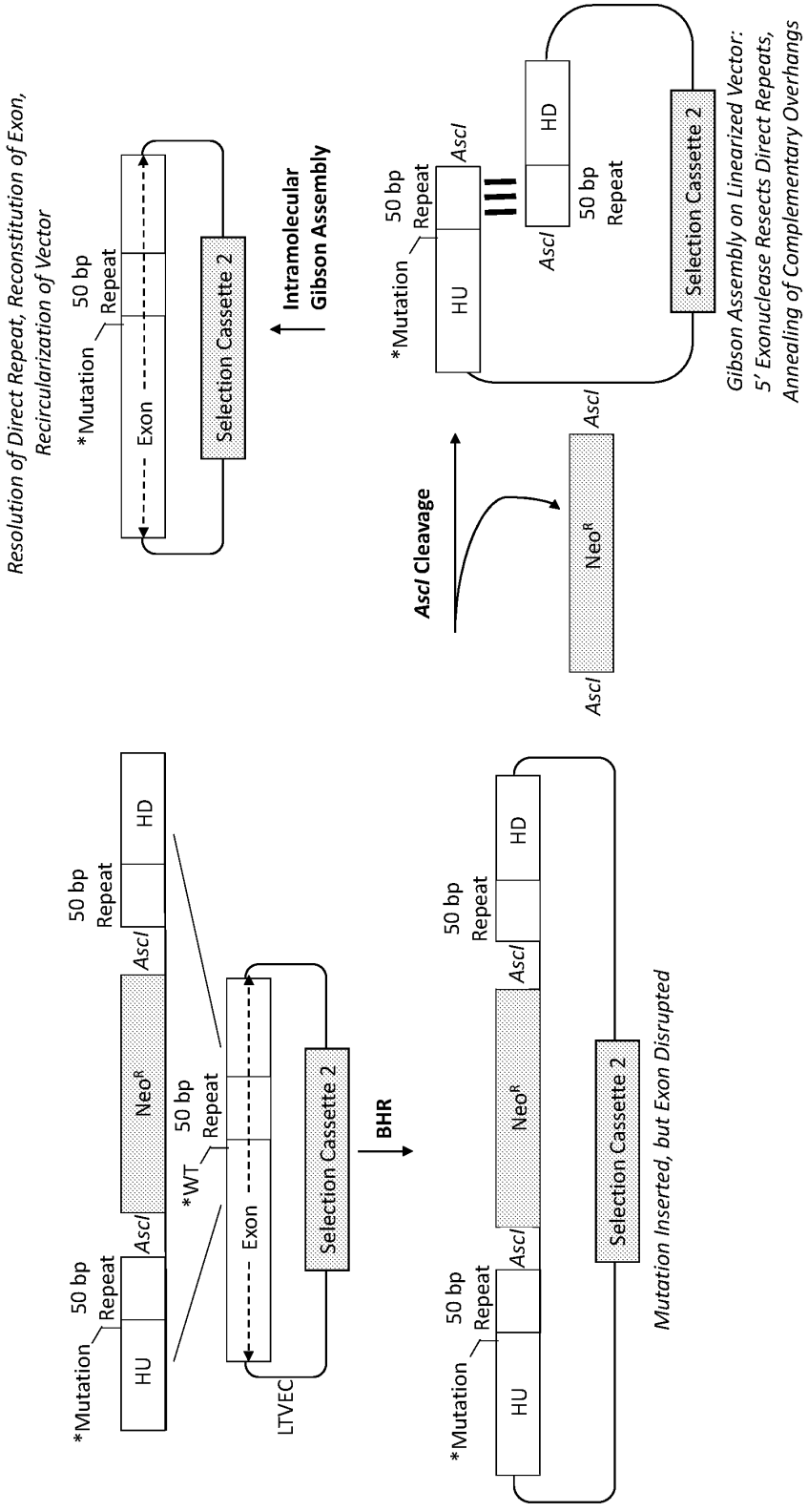

Resolution of Direct Repeat, Reconstitution of Exon, Recircularization of Vector
*Mutation
50 bp Repeat
Exon
Selection Cassette 2
Intramolecular Gibson Assembly
*Mutation
50 bp Repeat
HU
AscI
Neo^R
HD
*WT
Exon
LTVEC
BHR
Mutation Inserted, but Exon Disrupted
AscI Cleavage
Gibson Assembly on Linearized Vector: 5' Exonuclease Resects Direct Repeats, Annealing of Complementary Overhangs

FIG. 1

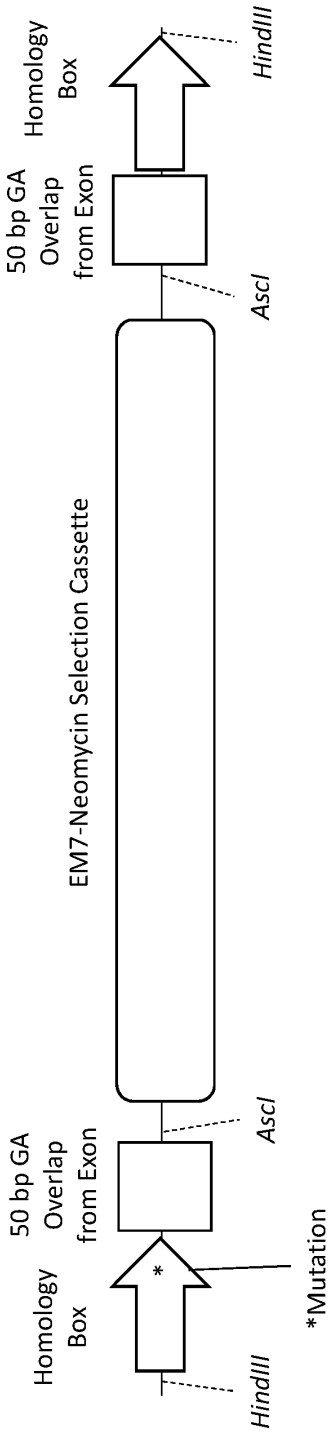

Homology Box
HindIII
50 bp GA Overlap from Exon
AscI
EM7-Neomycin Selection Cassette
AscI
50 bp GA Overlap from Exon
Homology Box
*
*Mutation
HindIII

FIG. 2

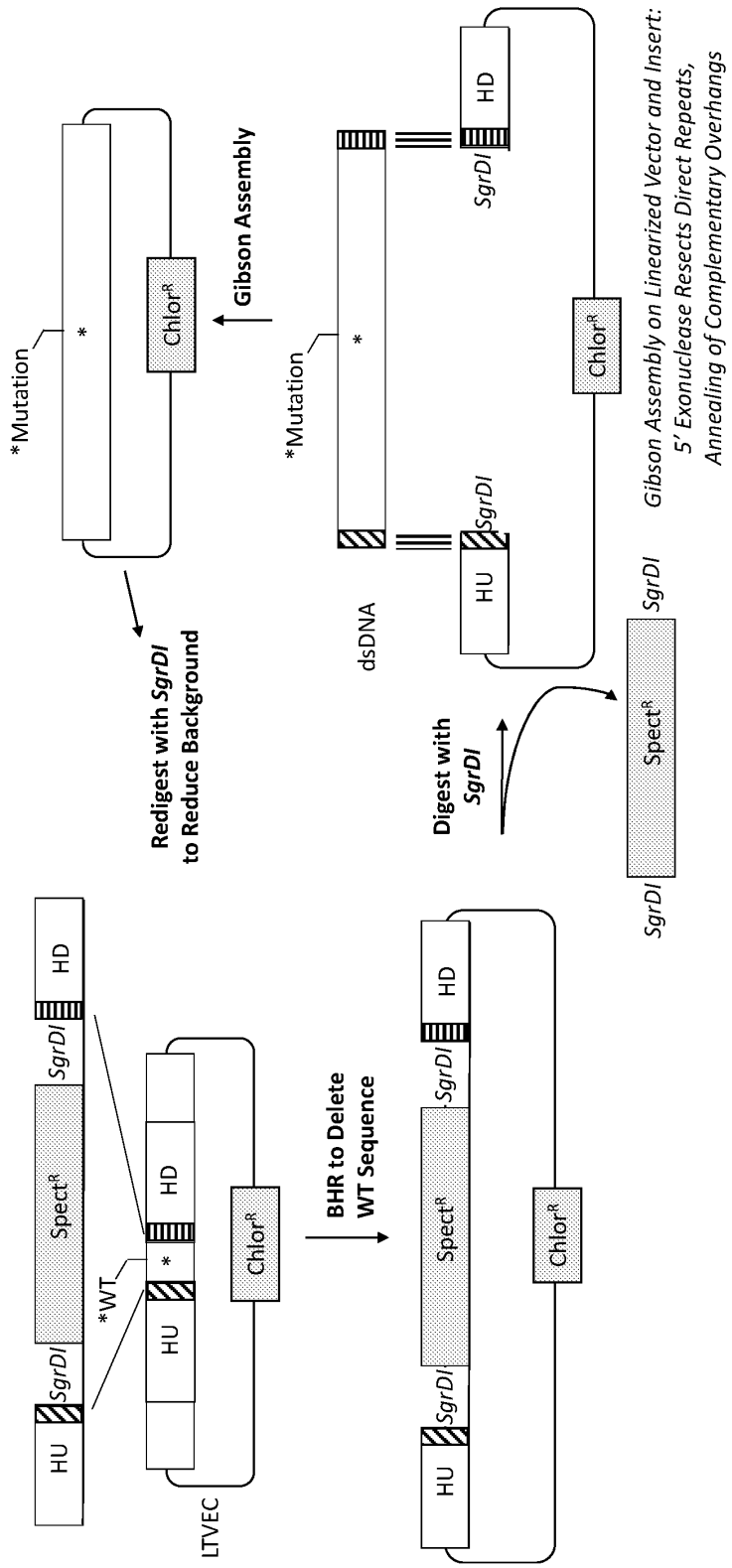

*Mutation
*
Chlor^R
Gibson Assembly
*Mutation
*
dsDNA
HU
SgrDI
SgrDI
HD
Chlor^R
Gibson Assembly on Linearized Vector and Insert:
5' Exonuclease Resects Direct Repeats,
Annealing of Complementary Overhangs
Digest with
SgrDI
SgrDI
Spect^R
SgrDI
Rediget with SgrDI
to Reduce Background
HU
SgrDI
Spect^R
SgrDI
HD
*WT
*
HU
HD
Chlor^R
LTVEC
BHR to Delete
WT Sequence
HU
SgrDI
Spect^R
SgrDI
HD
Chlor^R

FIG. 3

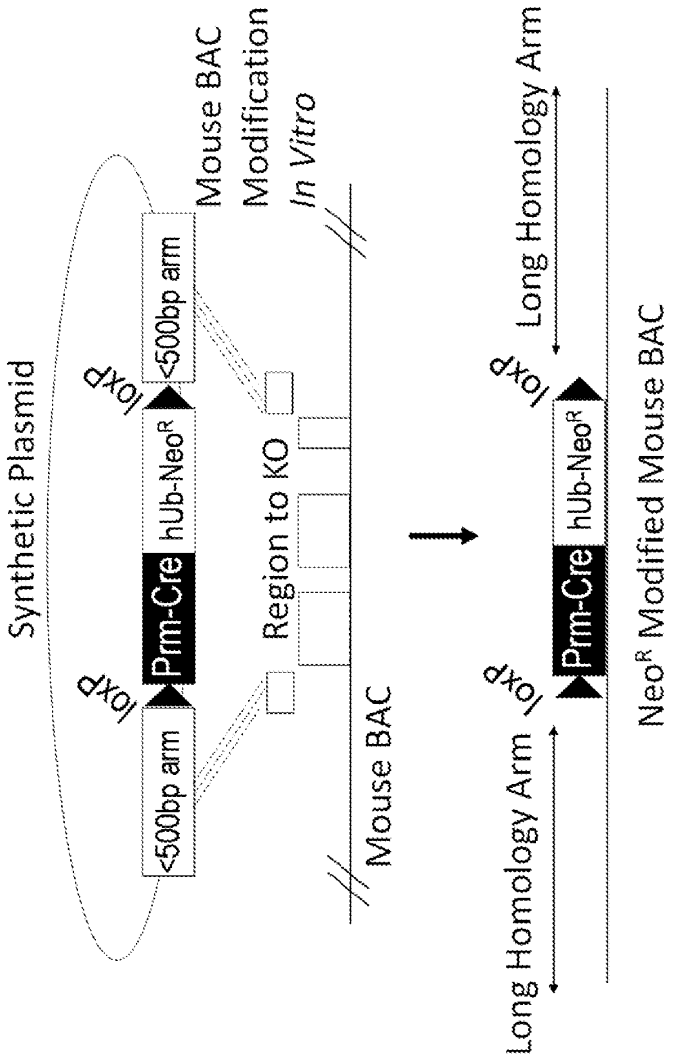

Synthetic Plasmid
<500bp arm
loxP
Prm-Cre
hUb-Neo^R
loxP
<500bp arm
Mouse BAC
Modification
In Vitro
Region to KO
Mouse BAC
Long Homology Arm
loxP
hUb-Neo^R
Prm-Cre
loxP
Long Homology Arm
Neo^R Modified Mouse BAC

FIG. 4A

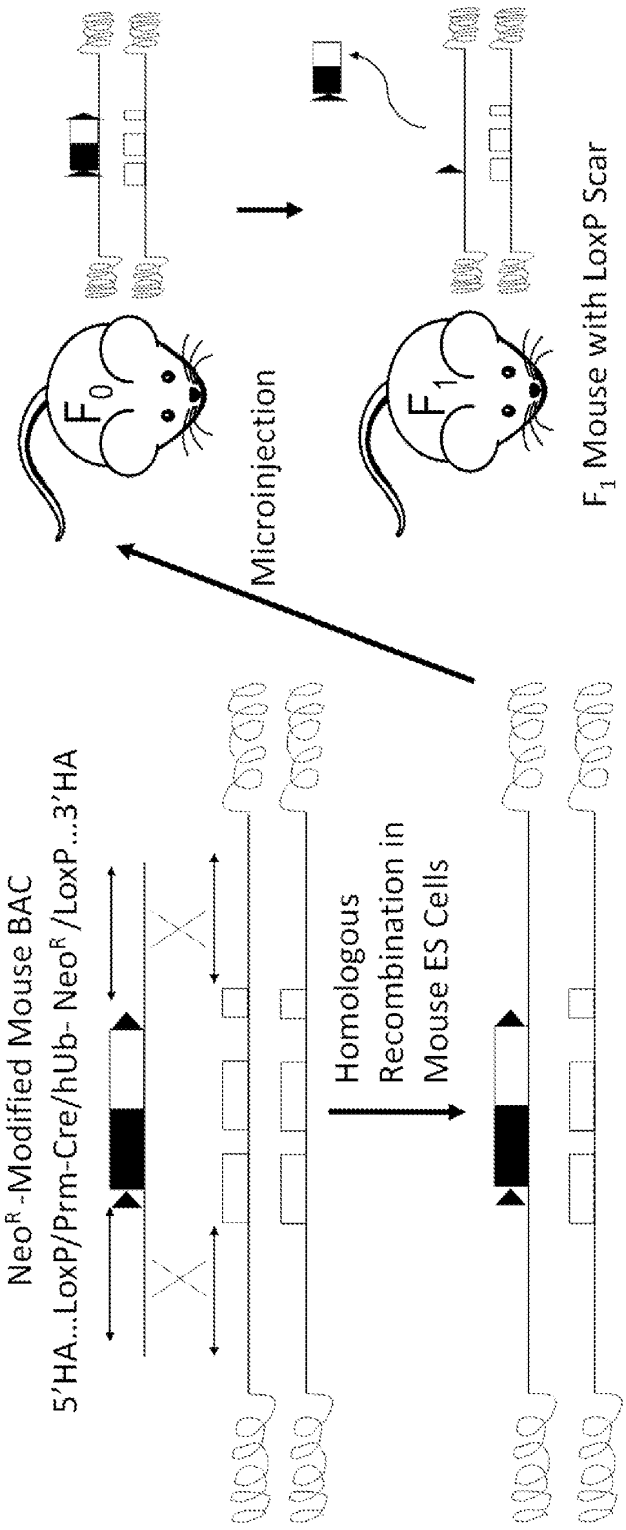

Neo^R-Modified Mouse BAC
5'HA...LoxP/Prm-Cre/hUb- Neo^R/LoxP...3'HA
Homologous
Recombination in
Mouse ES Cells
Microinjection
F_0
F_1
F_1 Mouse with LoxP Scar

FIG. 4B