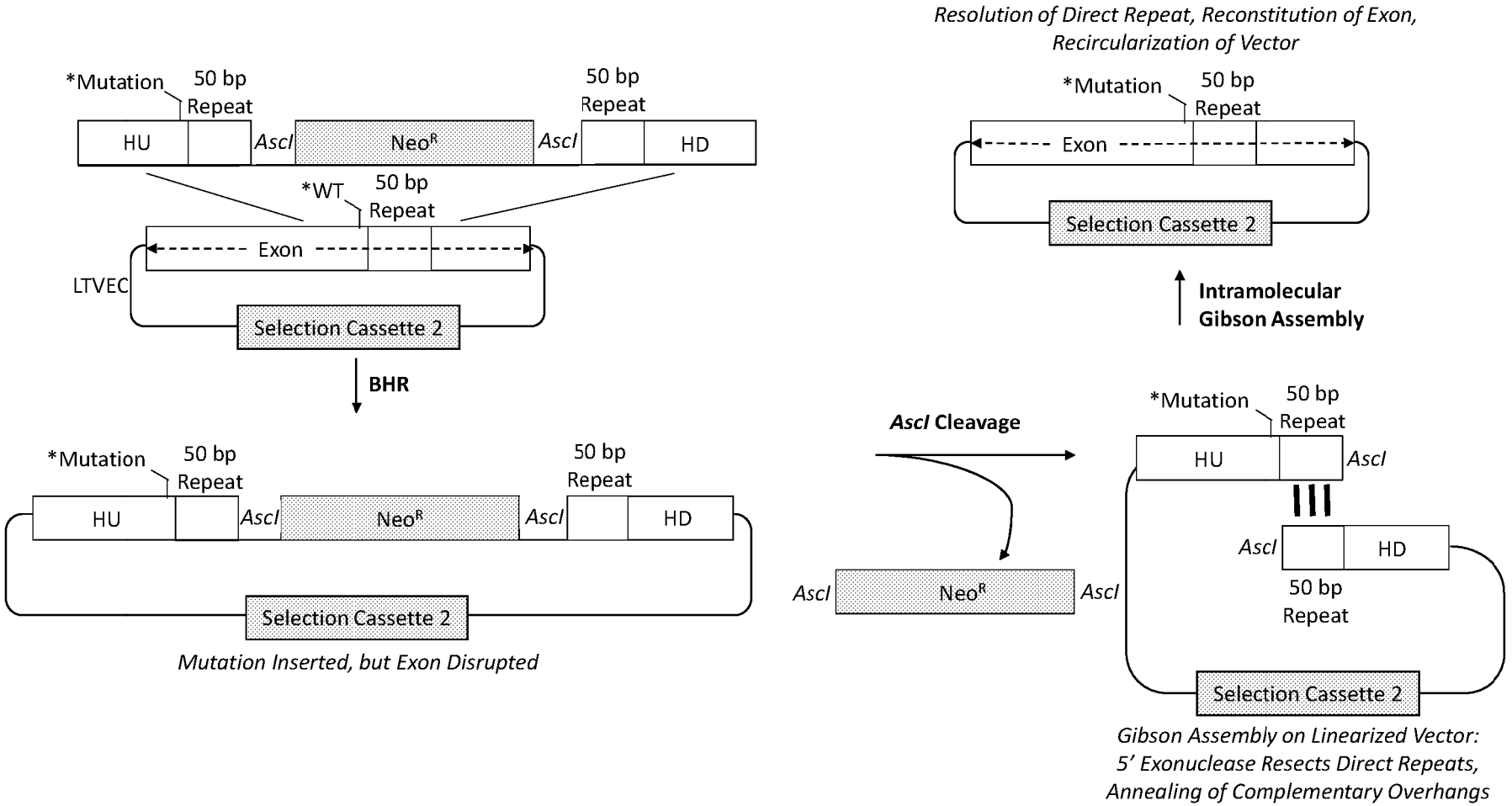

*Mutation
50 bp Repeat
HU
AscI
Neo^R
AscI
50 bp Repeat
HD
*WT
50 bp Repeat
Exon
LTVEC
Selection Cassette 2
BHR
*Mutation
50 bp Repeat
HU
AscI
Neo^R
AscI
50 bp Repeat
HD
Selection Cassette 2
Mutation Inserted, but Exon Disrupted
Resolution of Direct Repeat, Reconstitution of Exon, Recircularization of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

FIG. 1

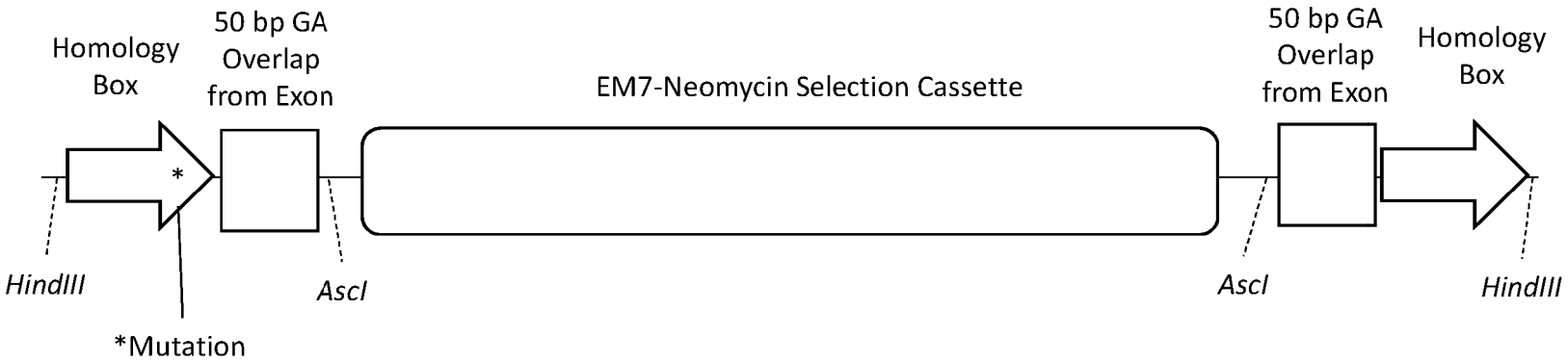

Homology Box
50 bp GA Overlap from Exon
EM7-Neomycin Selection Cassette
50 bp GA Overlap from Exon
Homology Box
*
HindIII
*Mutation
AscI
AscI
HindIII

FIG. 2

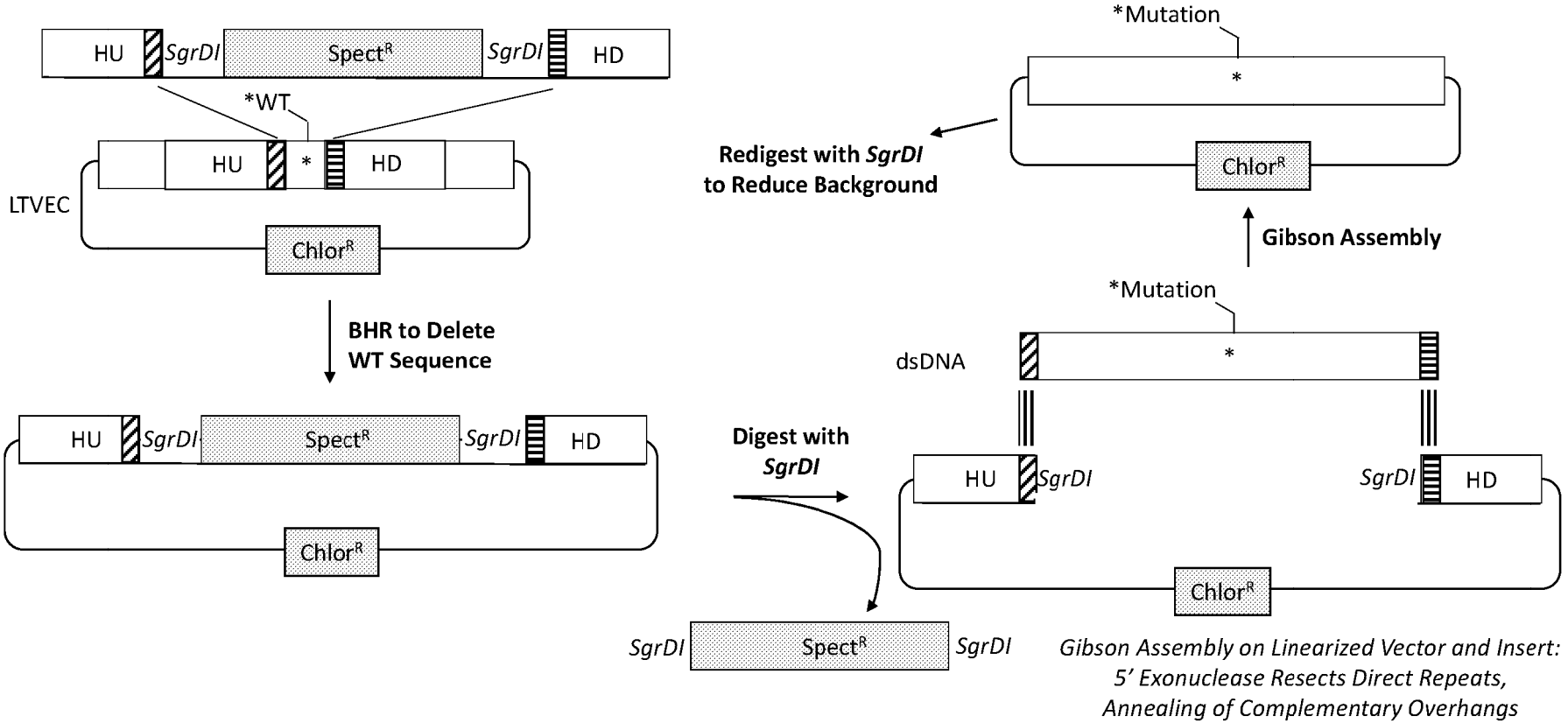

HU
SgrDI
Spect^R
SgrDI
HD
*WT
HU
*
HD
LTVEC
Chlor^R
BHR to Delete
WT Sequence
HU
SgrDI
Spect^R
SgrDI
HD
Chlor^R
*Mutation
*
Chlor^R
Redigest with SgrDI
to Reduce Background
Gibson Assembly
*Mutation
dsDNA
*
Digest with
SgrDI
HU
SgrDI
SgrDI
HD
Chlor^R
SgrDI
Spect^R
SgrDI
Gibson Assembly on Linearized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

FIG. 3

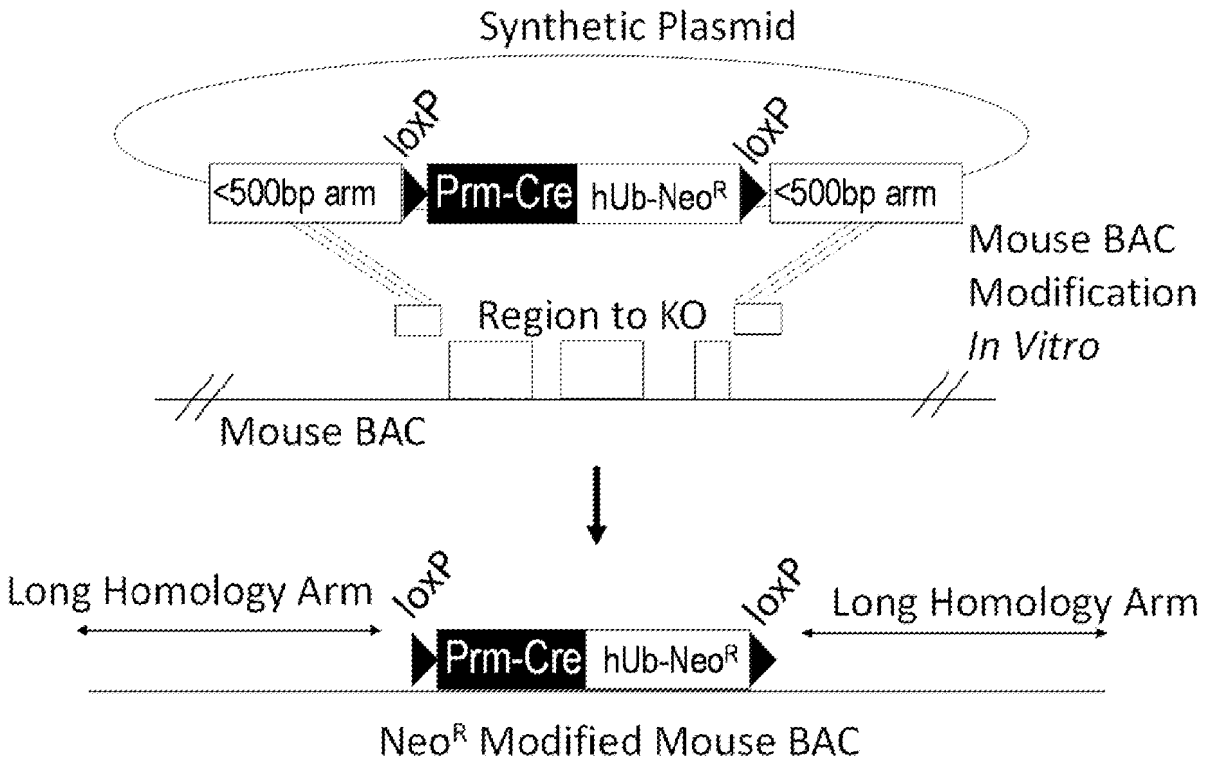

Synthetic Plasmid
loxP
loxP
<500bp arm
Prm-Cre
hUb-Neo^R
<500bp arm
Mouse BAC
Modification
In Vitro
Region to KO
Mouse BAC
Long Homology Arm
loxP
loxP
Long Homology Arm
Prm-Cre
hUb-Neo^R
Neo^R Modified Mouse BAC

FIG. 4A

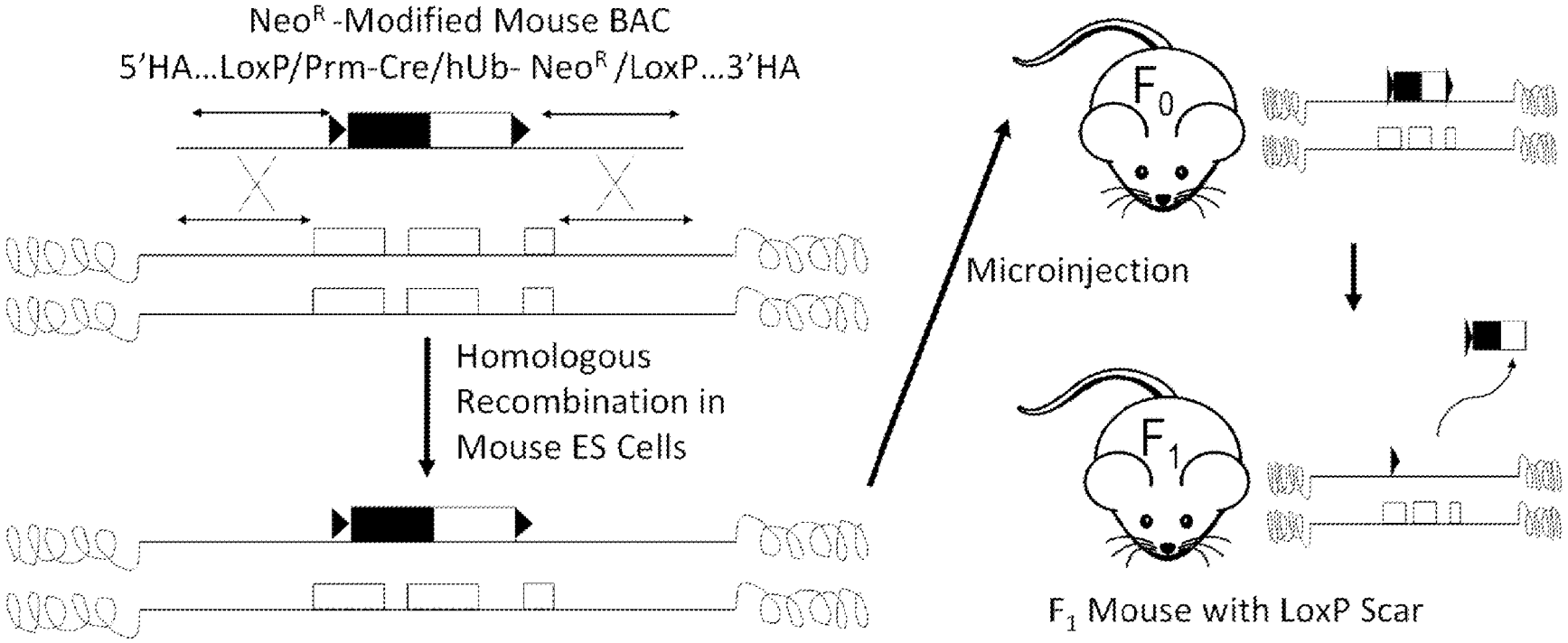

Neo^R -Modified Mouse BAC
5'HA...LoxP/Prm-Cre/hUb- Neo^R /LoxP...3'HA
Homologous
Recombination in
Mouse ES Cells
Microinjection
F_0
F_1
F_1 Mouse with LoxP Scar

FIG. 4B