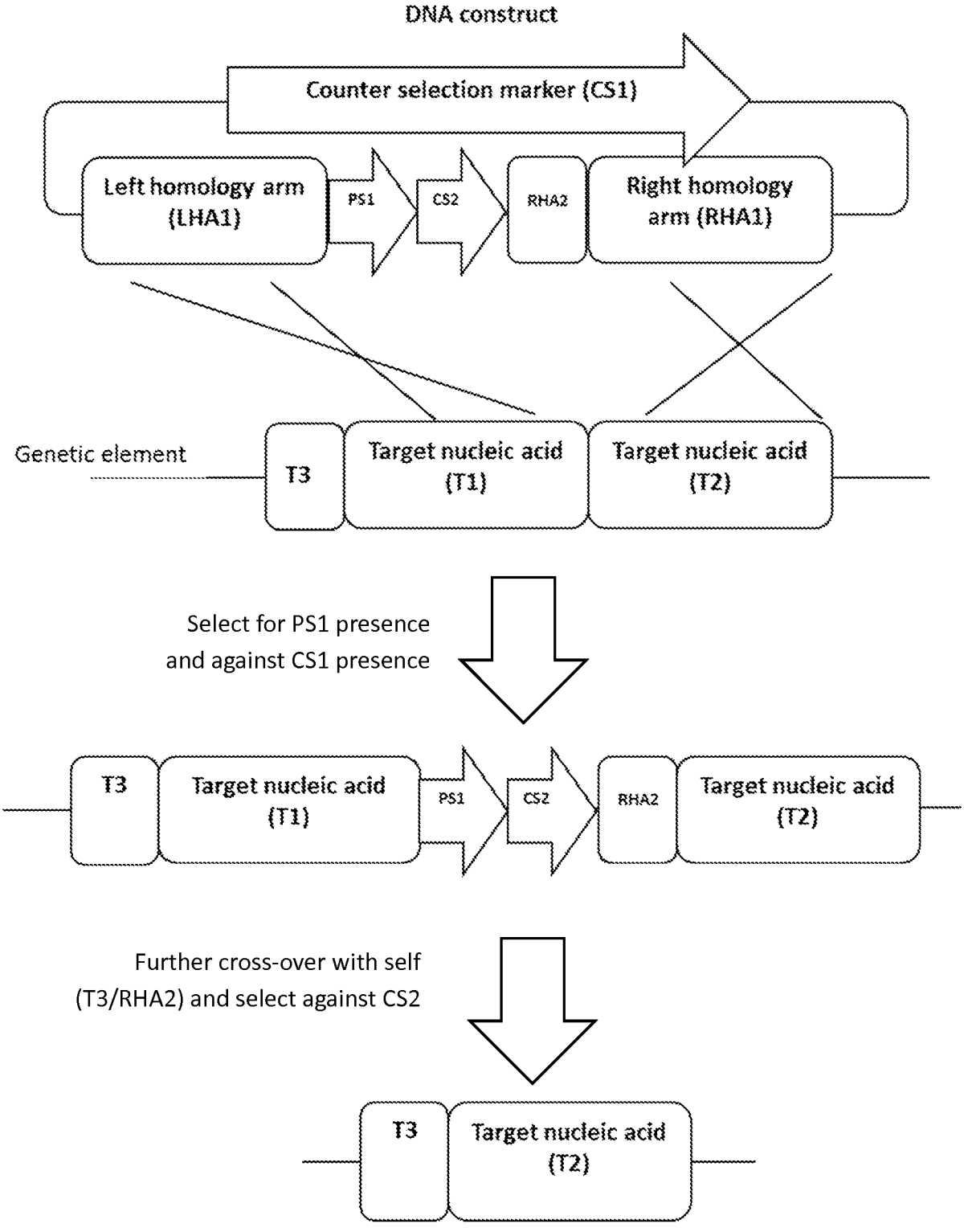

DNA construct
Counter selection marker (CS1)
Left homology arm (LHA1)
PS1
CS2
RHA2
Right homology arm (RHA1)
Genetic element
T3
Target nucleic acid (T1)
Target nucleic acid (T2)
Select for PS1 presence and against CS1 presence
T3
Target nucleic acid (T1)
PS1
CS2
RHA2
Target nucleic acid (T2)
Further cross-over with self (T3/RHA2) and select against CS2
T3
Target nucleic acid (T2)

FIG. 1

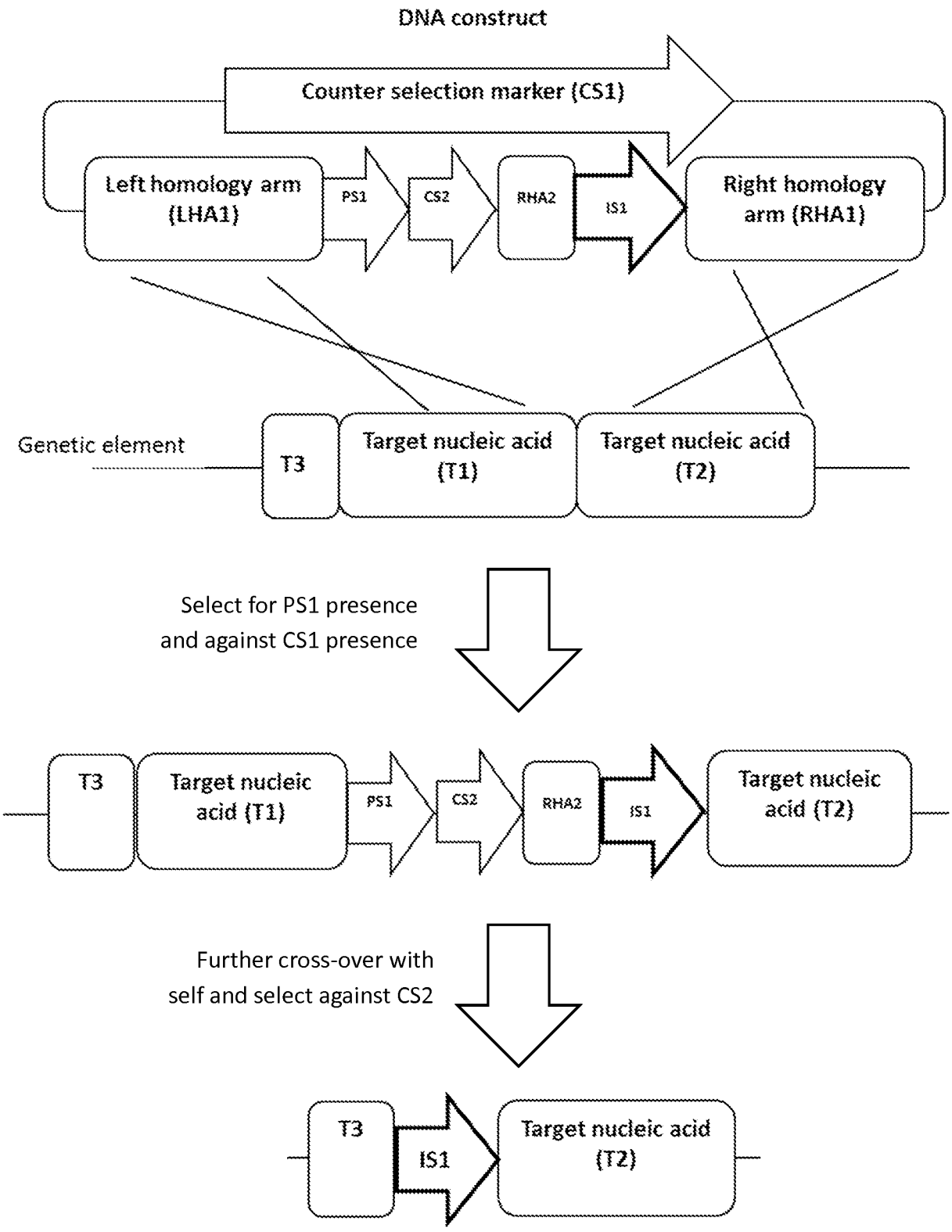

DNA construct
Counter selection marker (CS1)
Left homology arm (LHA1)
PS1
CS2
RHA2
IS1
Right homology arm (RHA1)
Genetic element
T3
Target nucleic acid (T1)
Target nucleic acid (T2)
Select for PS1 presence and against CS1 presence
T3
Target nucleic acid (T1)
PS1
CS2
RHA2
IS1
Target nucleic acid (T2)
Further cross-over with self and select against CS2
T3
IS1
Target nucleic acid (T2)

FIG. 2

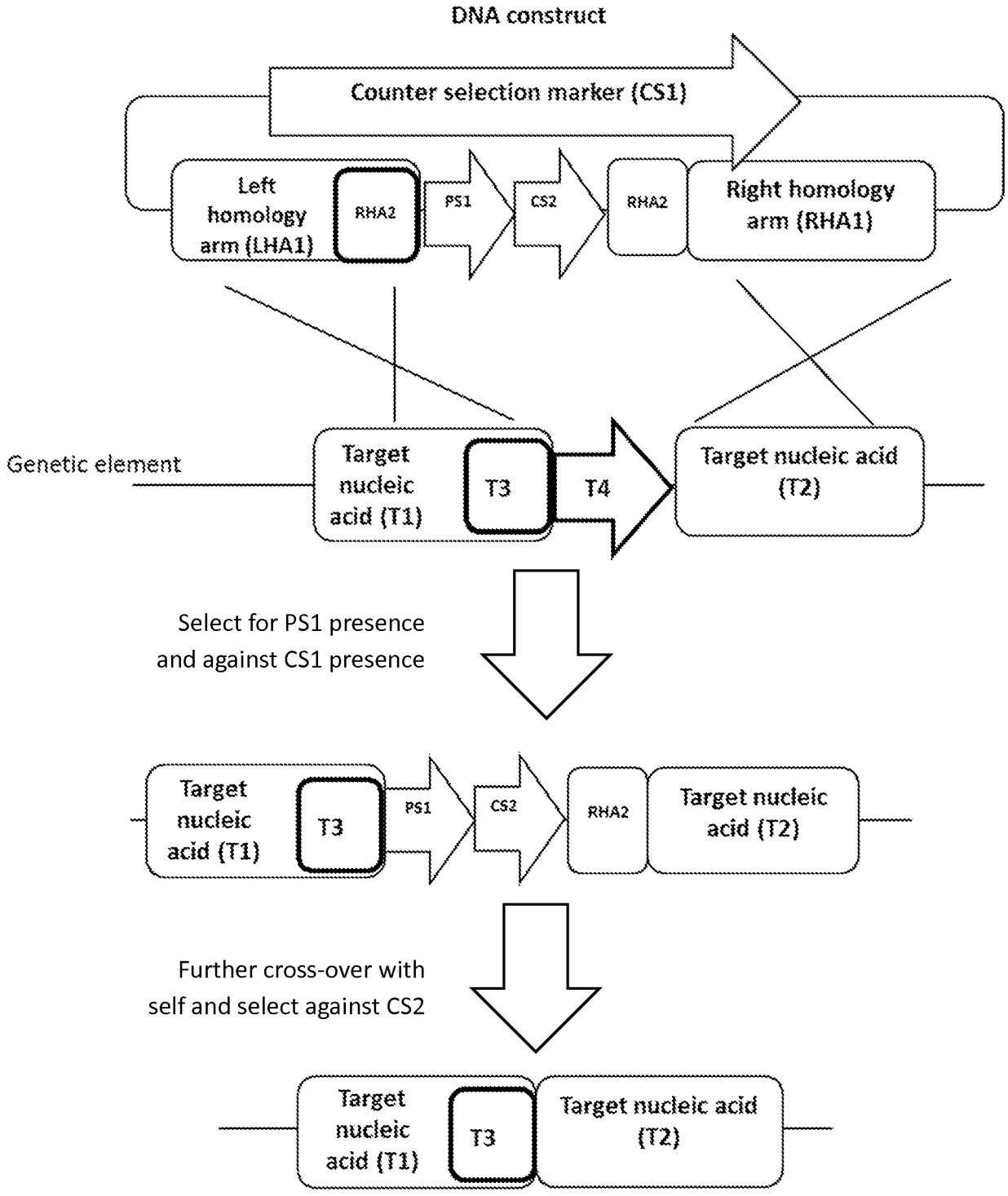

DNA construct
Counter selection marker (CS1)
Left homology arm (LHA1)
RHA2
PS1
CS2
RHA2
Right homology arm (RHA1)
Genetic element
Target nucleic acid (T1)
T3
T4
Target nucleic acid (T2)
Select for PS1 presence and against CS1 presence
Target nucleic acid (T1)
T3
PS1
CS2
RHA2
Target nucleic acid (T2)
Further cross-over with self and select against CS2
Target nucleic acid (T1)
T3
Target nucleic acid (T2)

FIG. 3

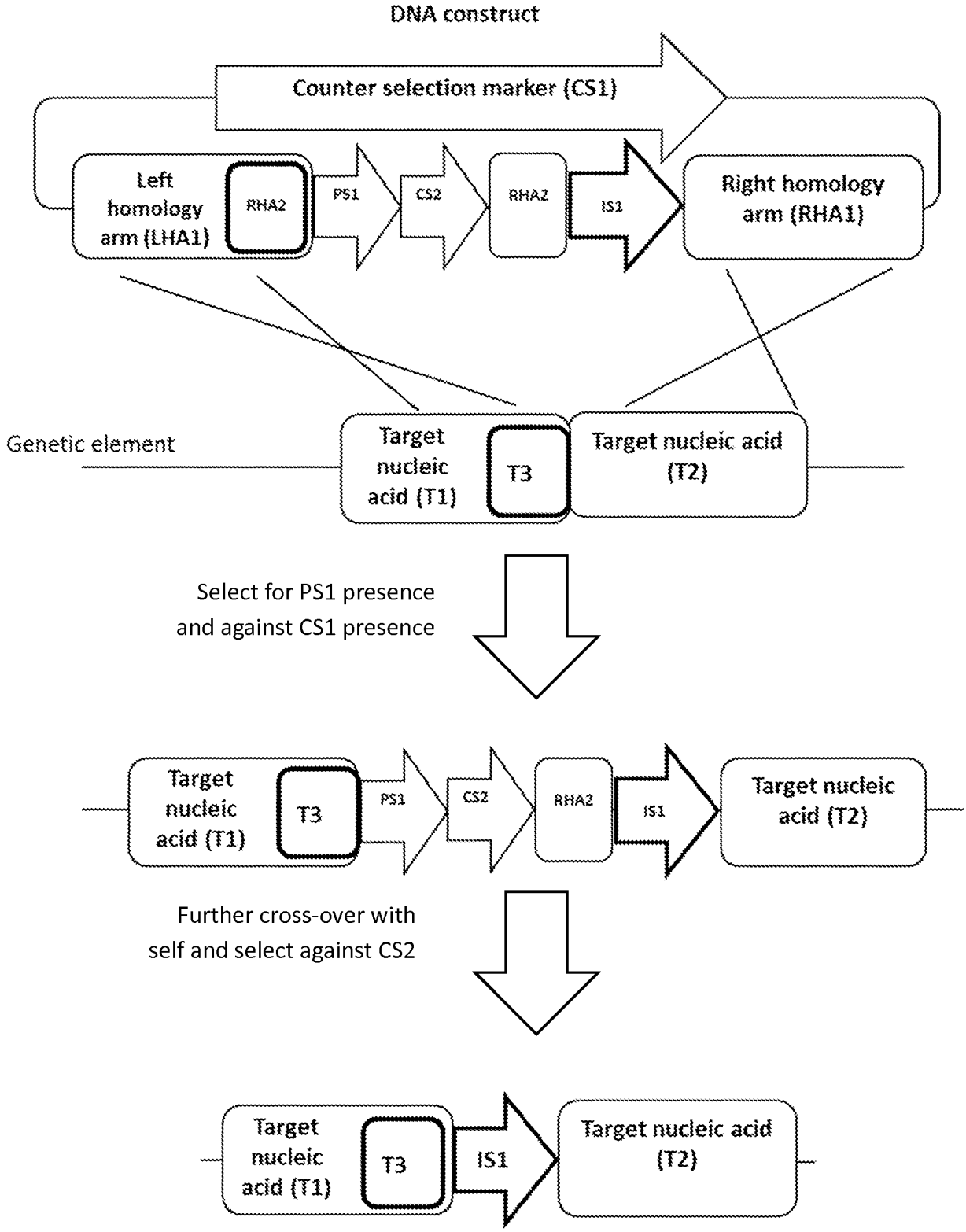

DNA construct
Counter selection marker (CS1)
Left homology arm (LHA1)
RHA2
PS1
CS2
RHA2
IS1
Right homology arm (RHA1)
Genetic element
Target nucleic acid (T1)
T3
Target nucleic acid (T2)
Select for PS1 presence and against CS1 presence
Target nucleic acid (T1)
T3
PS1
CS2
RHA2
IS1
Target nucleic acid (T2)
Further cross-over with self and select against CS2
Target nucleic acid (T1)
T3
IS1
Target nucleic acid (T2)

FIG. 4

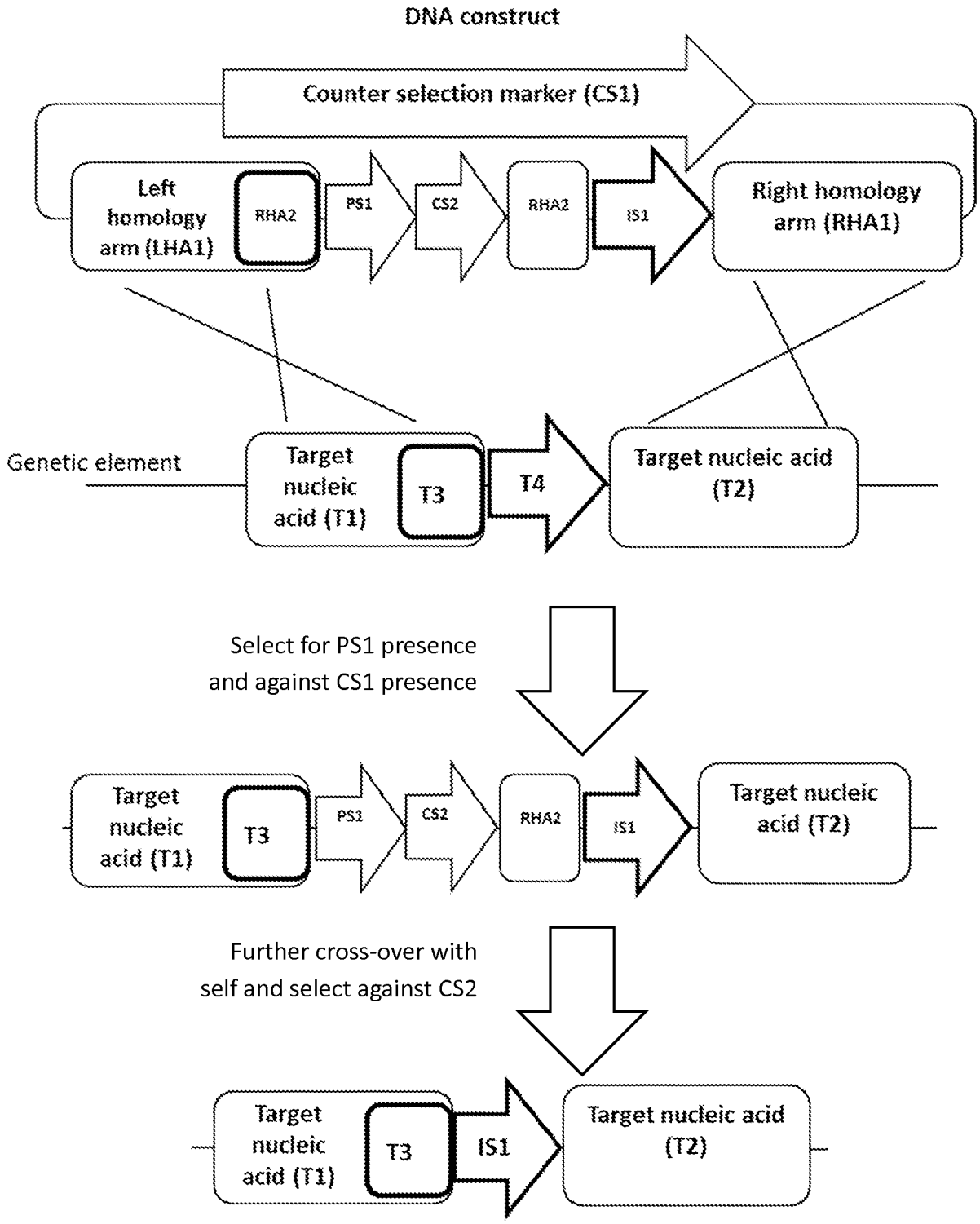

DNA construct
Counter selection marker (CS1)
Left homology arm (LHA1)
RHA2
PS1
CS2
RHA2
IS1
Right homology arm (RHA1)
Genetic element
Target nucleic acid (T1)
T3
T4
Target nucleic acid (T2)
Select for PS1 presence and against CS1 presence
Target nucleic acid (T1)
T3
PS1
CS2
RHA2
IS1
Target nucleic acid (T2)
Further cross-over with self and select against CS2
Target nucleic acid (T1)
T3
IS1
Target nucleic acid (T2)

FIG. 5

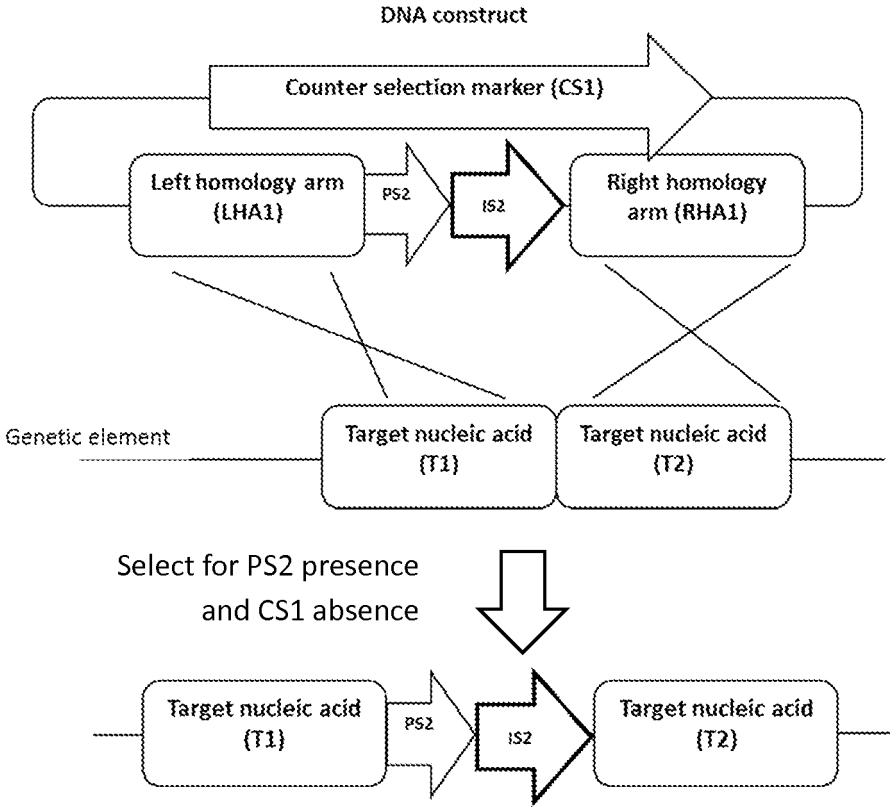

DNA construct
Counter selection marker (CS1)
Left homology arm (LHA1)
PS2
IS2
Right homology arm (RHA1)
Genetic element
Target nucleic acid (T1)
Target nucleic acid (T2)
Select for PS2 presence and CS1 absence
Target nucleic acid (T1)
PS2
IS2
Target nucleic acid (T2)

FIG. 6A

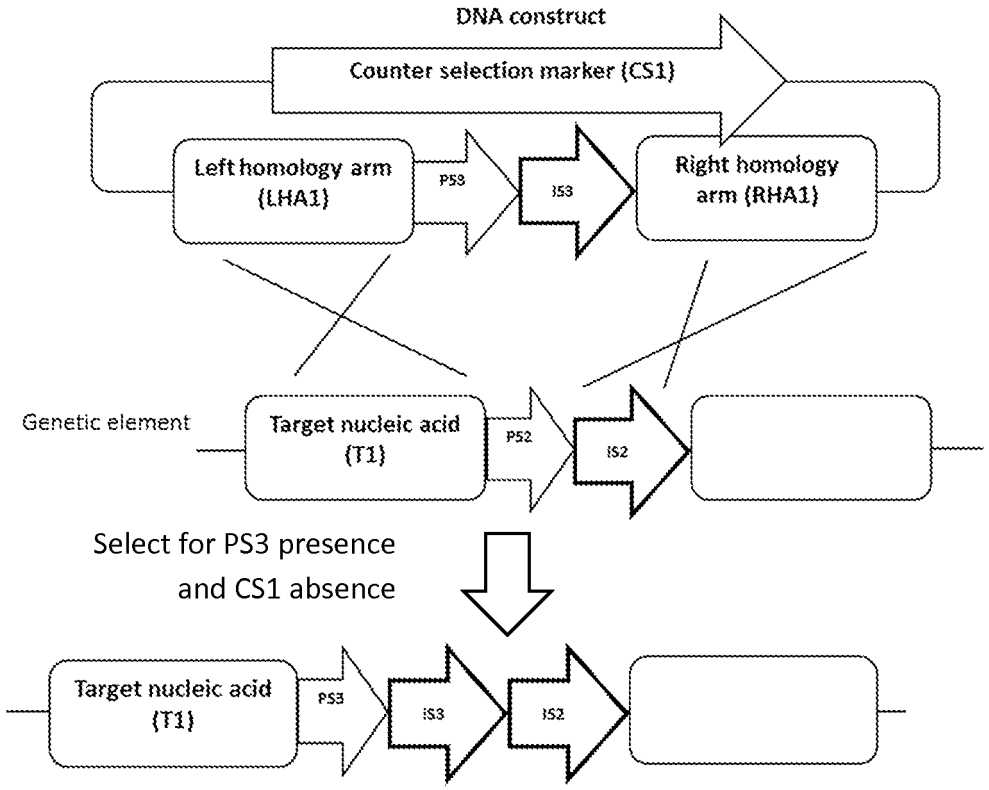

DNA construct
Counter selection marker (CS1)
Left homology arm (LHA1)
PS3
IS3
Right homology arm (RHA1)
Genetic element
Target nucleic acid (T1)
PS2
IS2
Select for PS3 presence and CS1 absence
Target nucleic acid (T1)
PS3
IS3
IS2

FIG. 6B

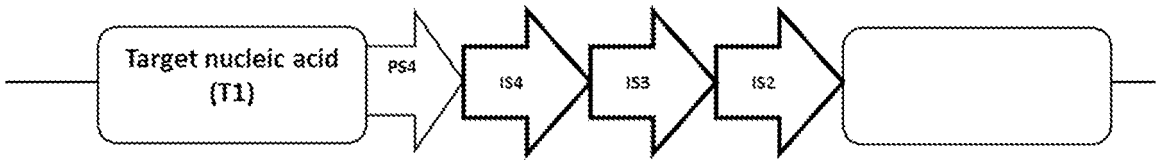

Target nucleic acid (T1)
PS4
IS4
IS3
IS2

FIG. 6C

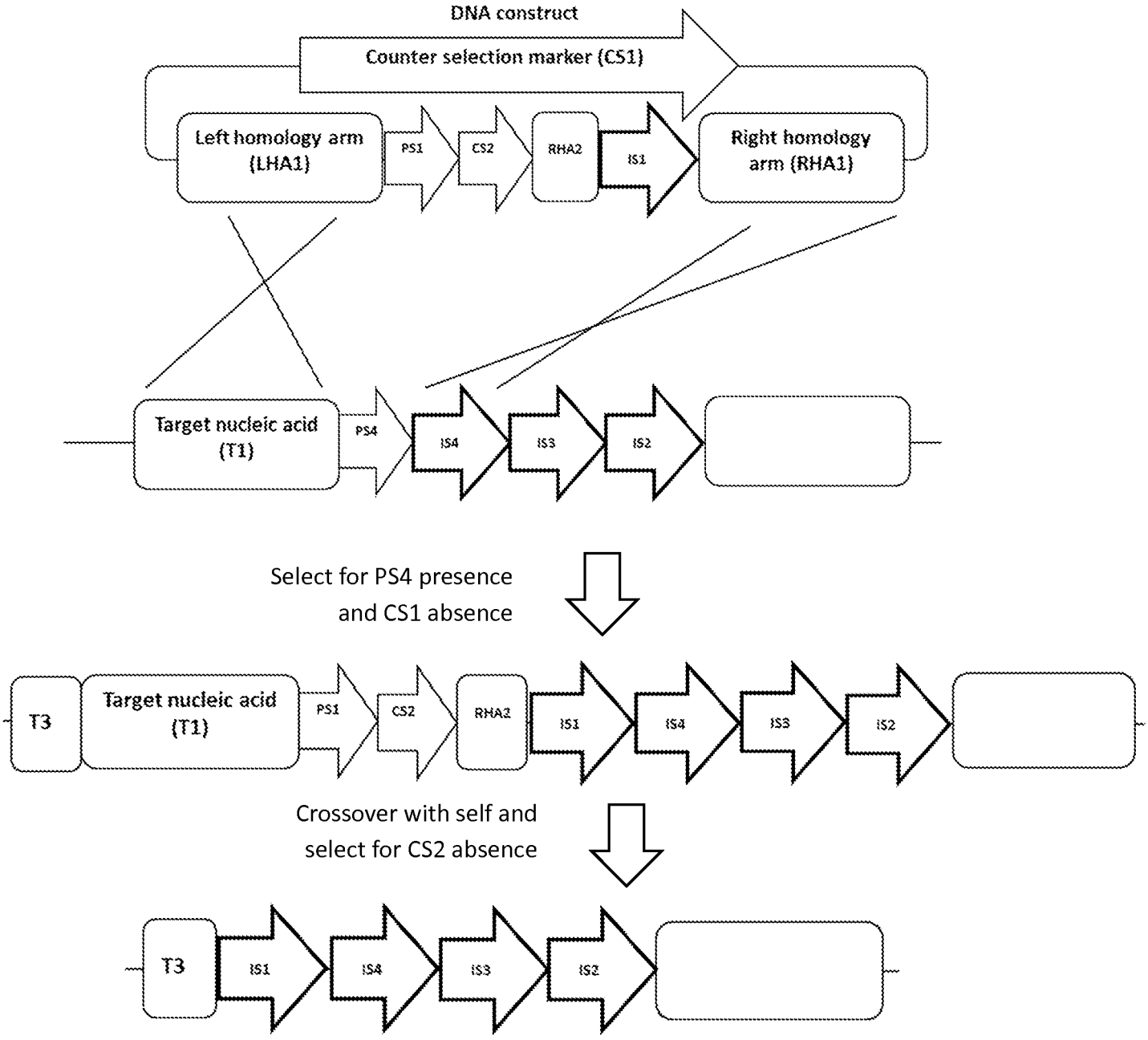

DNA construct
Counter selection marker (CS1)
Left homology arm (LHA1)
PS1
CS2
RHA2
IS1
Right homology arm (RHA1)
Target nucleic acid (T1)
PS4
IS4
IS3
IS2
Select for PS4 presence and CS1 absence
T3
Target nucleic acid (T1)
PS1
CS2
RHA2
IS1
IS4
IS3
IS2
Crossover with self and select for CS2 absence
T3
IS1
IS4
IS3
IS2

FIG. 6D

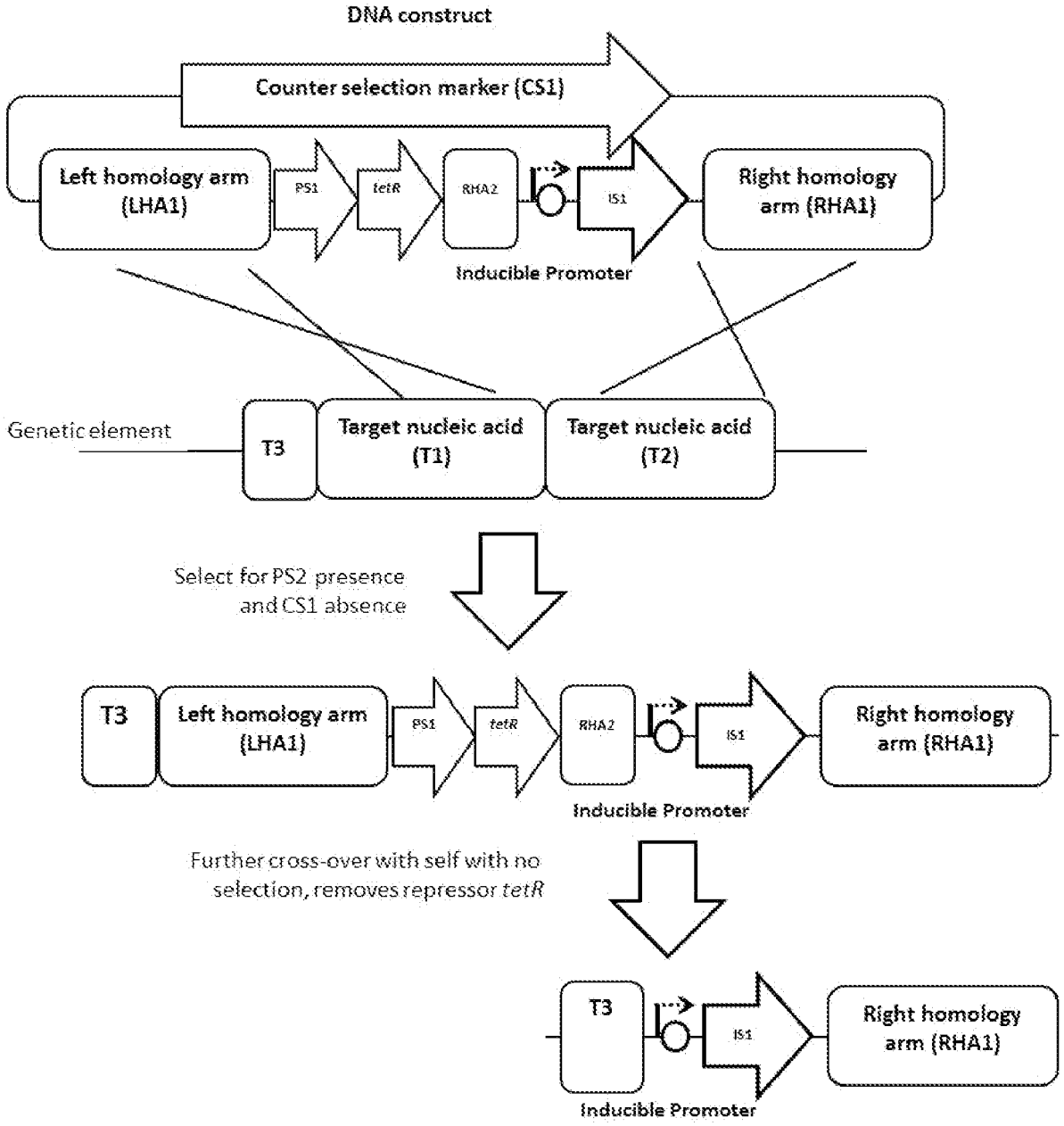

DNA construct
Counter selection marker (CS1)
Left homology arm (LHA1)
PS1
tetR
RHA2
IS1
Right homology arm (RHA1)
Inducible Promoter
Genetic element
T3
Target nucleic acid (T1)
Target nucleic acid (T2)
Select for PS2 presence and CS1 absence
T3
Left homology arm (LHA1)
PS1
tetR
RHA2
IS1
Right homology arm (RHA1)
Inducible Promoter
Further cross-over with self with no selection, removes repressor tetR
T3
IS1
Right homology arm (RHA1)
Inducible Promoter

FIG. 7

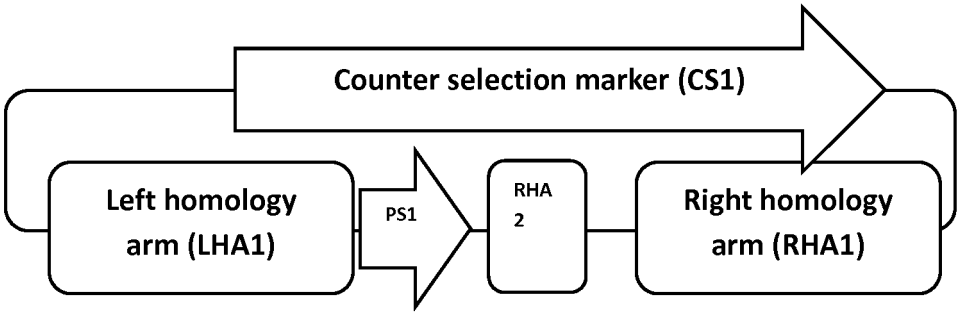

Counter selection marker (CS1)
Left homology arm (LHA1)
PS1
RHA 2
Right homology arm (RHA1)

FIG. 8

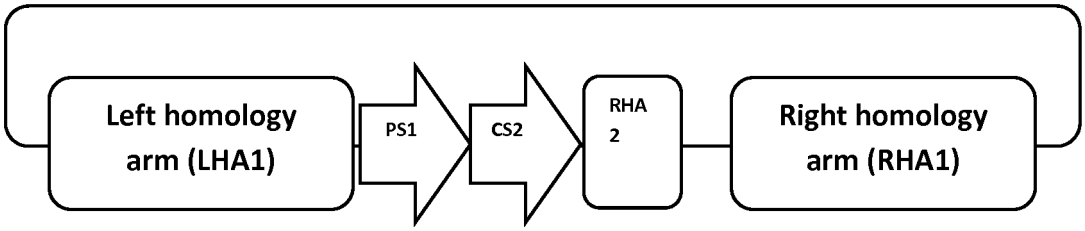


FIG. 9

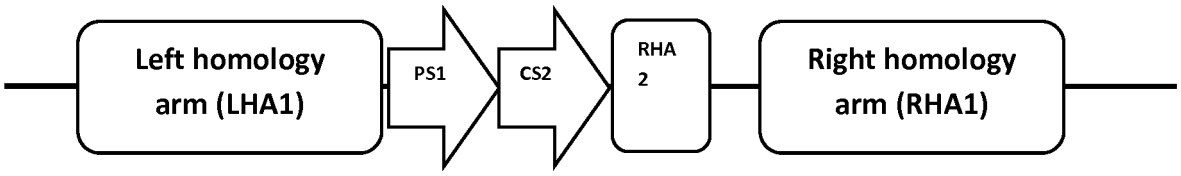


FIG. 10

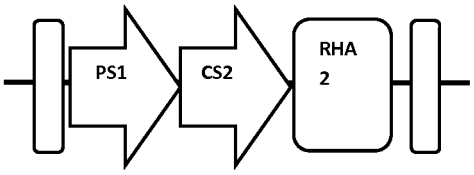


FIG. 11

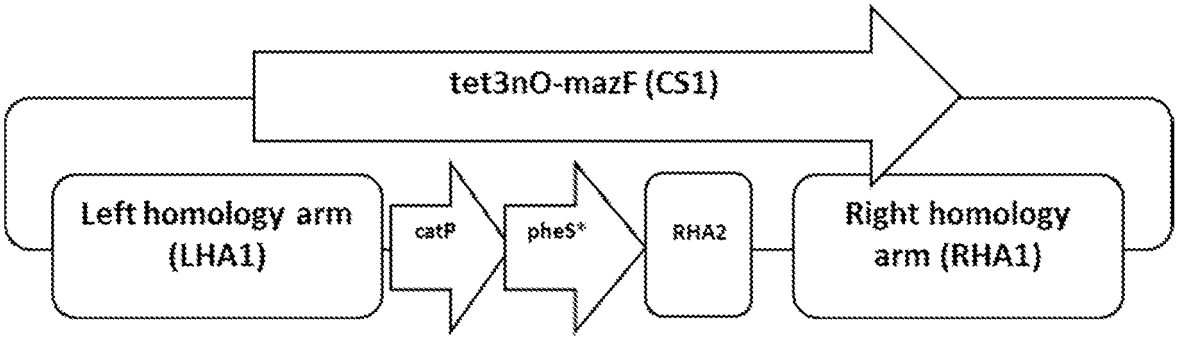


FIG. 12

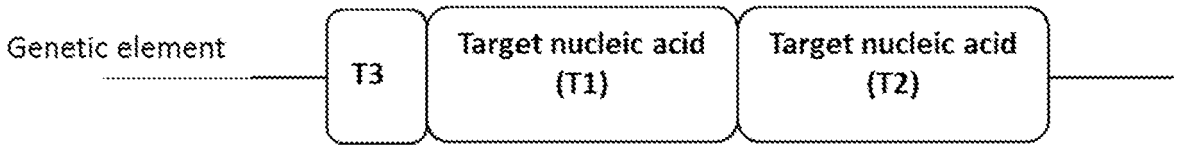


FIG. 13

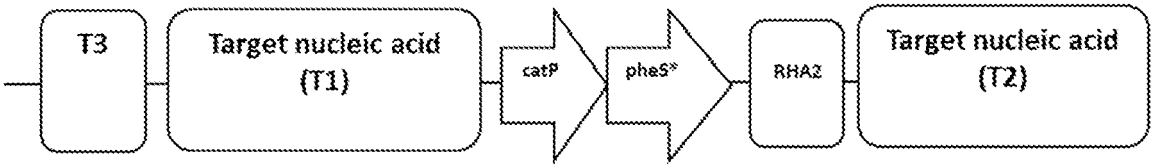


FIG. 14

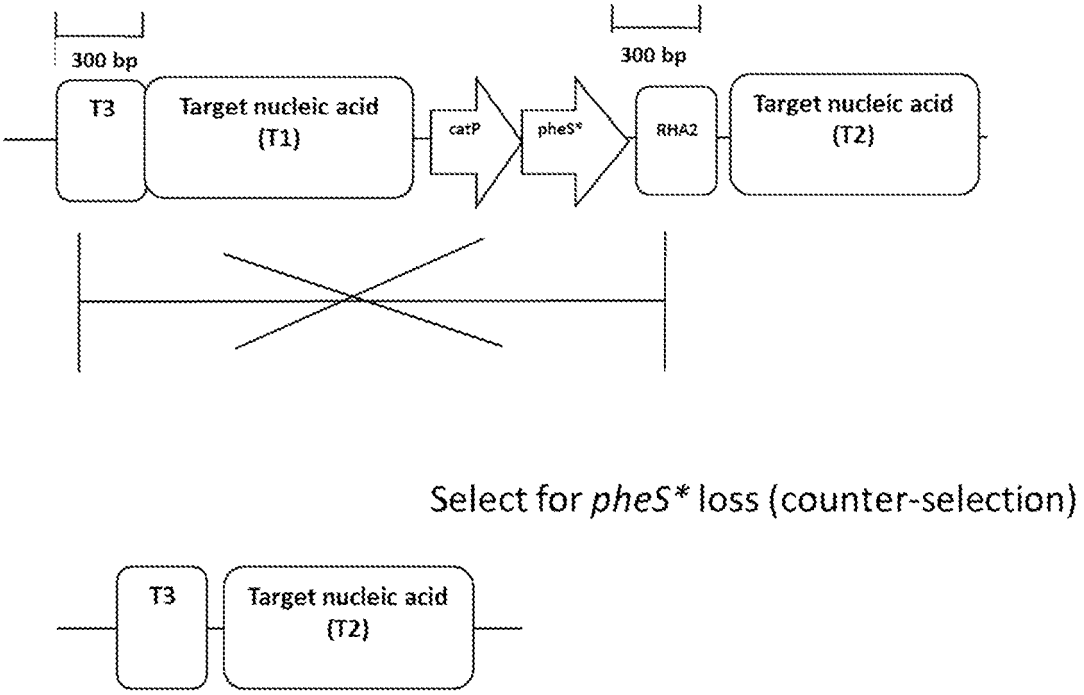

300 bp
300 bp
T3
Target nucleic acid (T1)
catP
pheS*
RHA2
Target nucleic acid (T2)
Select for pheS* loss (counter-selection)
T3
Target nucleic acid (T2)

FIG. 15

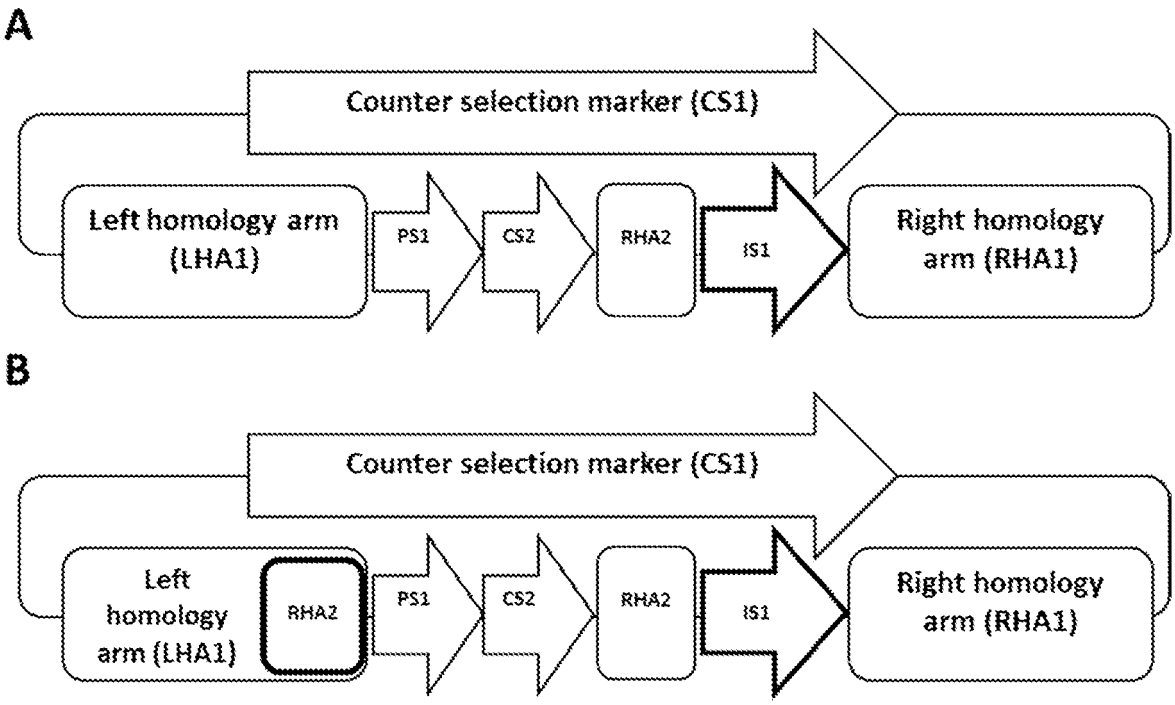

A
Counter selection marker (CS1)
Left homology arm (LHA1)
PS1
CS2
RHA2
IS1
Right homology arm (RHA1)
B
Counter selection marker (CS1)
Left homology arm (LHA1)
RHA2
PS1
CS2
RHA2
IS1
Right homology arm (RHA1)

FIG. 16

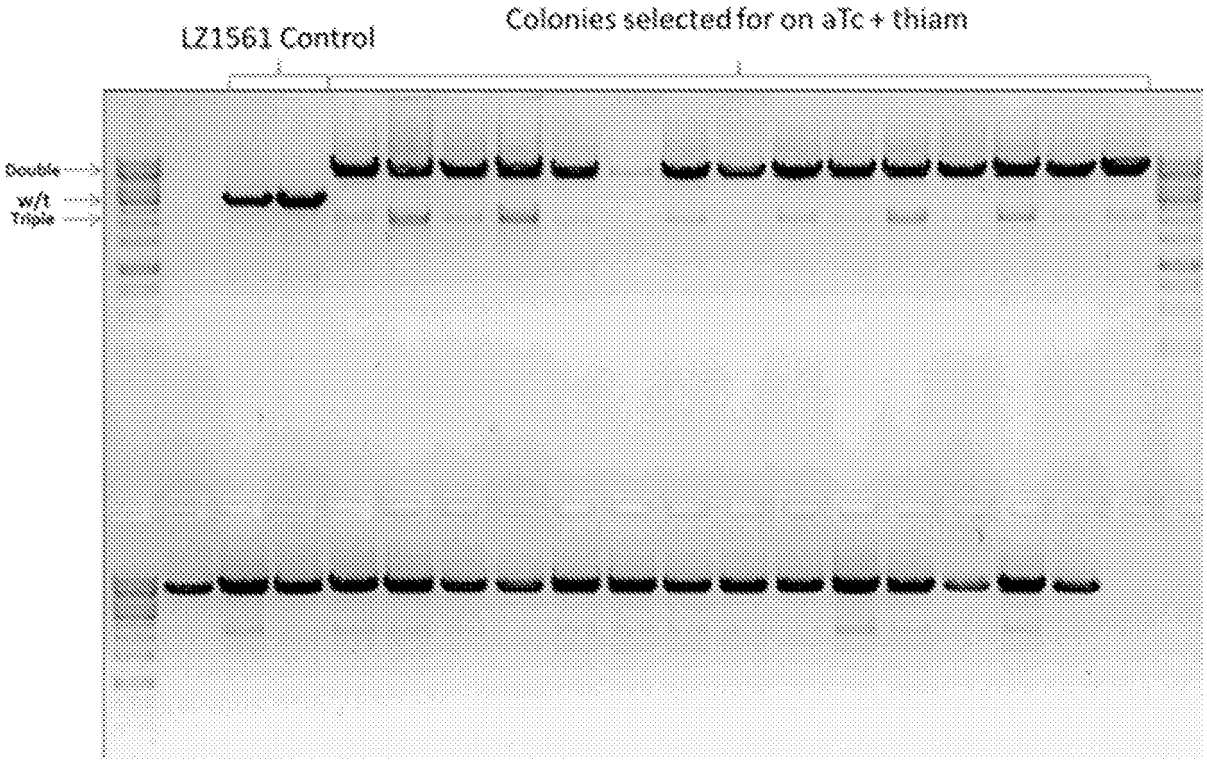

LZ1561 Control
Colonies selected for on aTc + thiam
Double
w/t
Triple

FIG. 17