FIG. 1

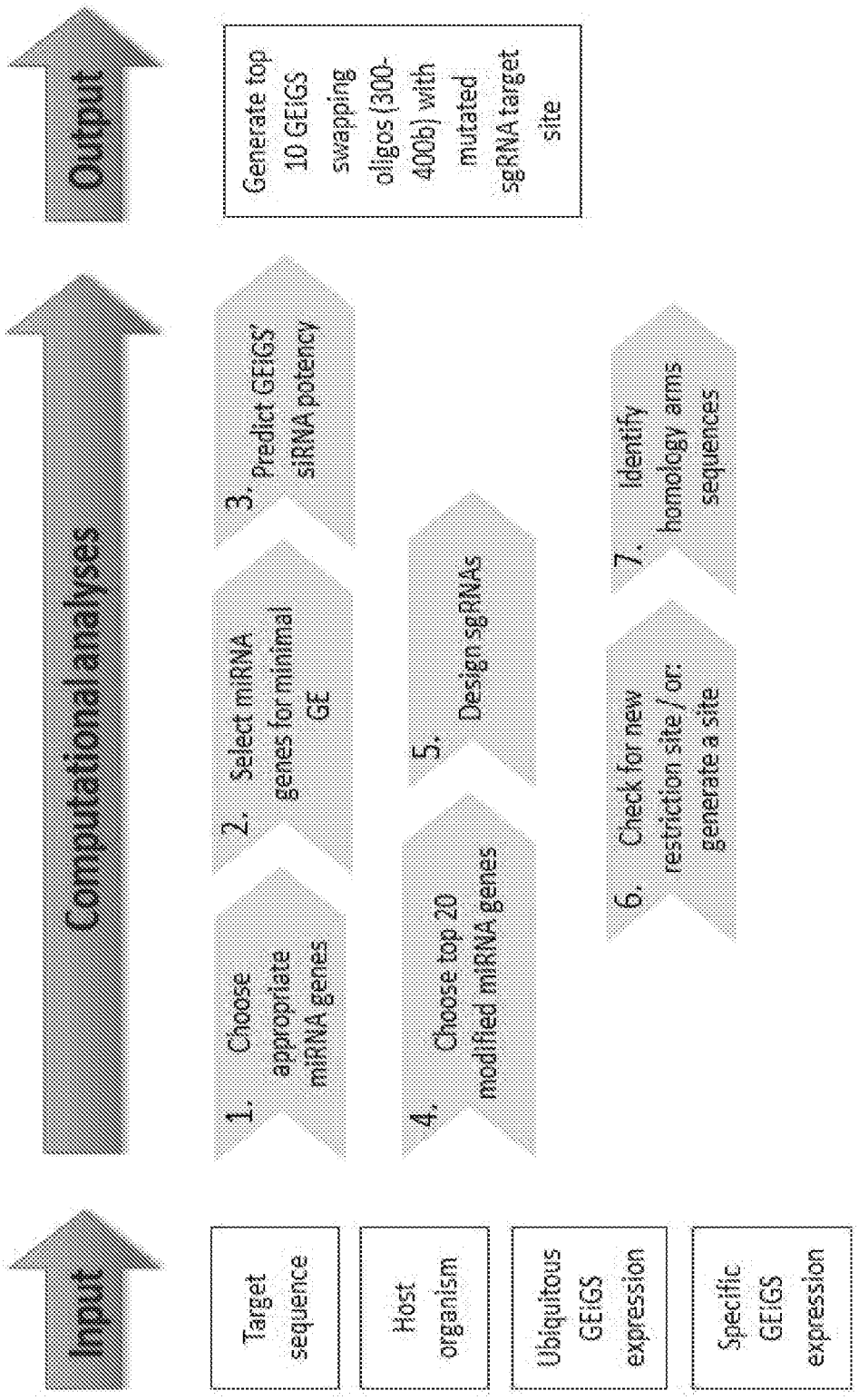

Input
Computational analyses
Output
Target sequence
Host organism
Ubiquitous GEiGS expression
Specific GEiGS expression
1. Choose appropriate miRNA genes
2. Select miRNA genes for minimal GE
3. Predict GEiGS' siRNA potency
4. Choose top 20 modified miRNA genes
5. Design sgRNAs
6. Check for new restriction site / or: generate a site
7. Identify homology arms sequences
Generate top 10 GEiGS swapping oligos (300-400b) with mutated sgRNA target site

FIG. 2

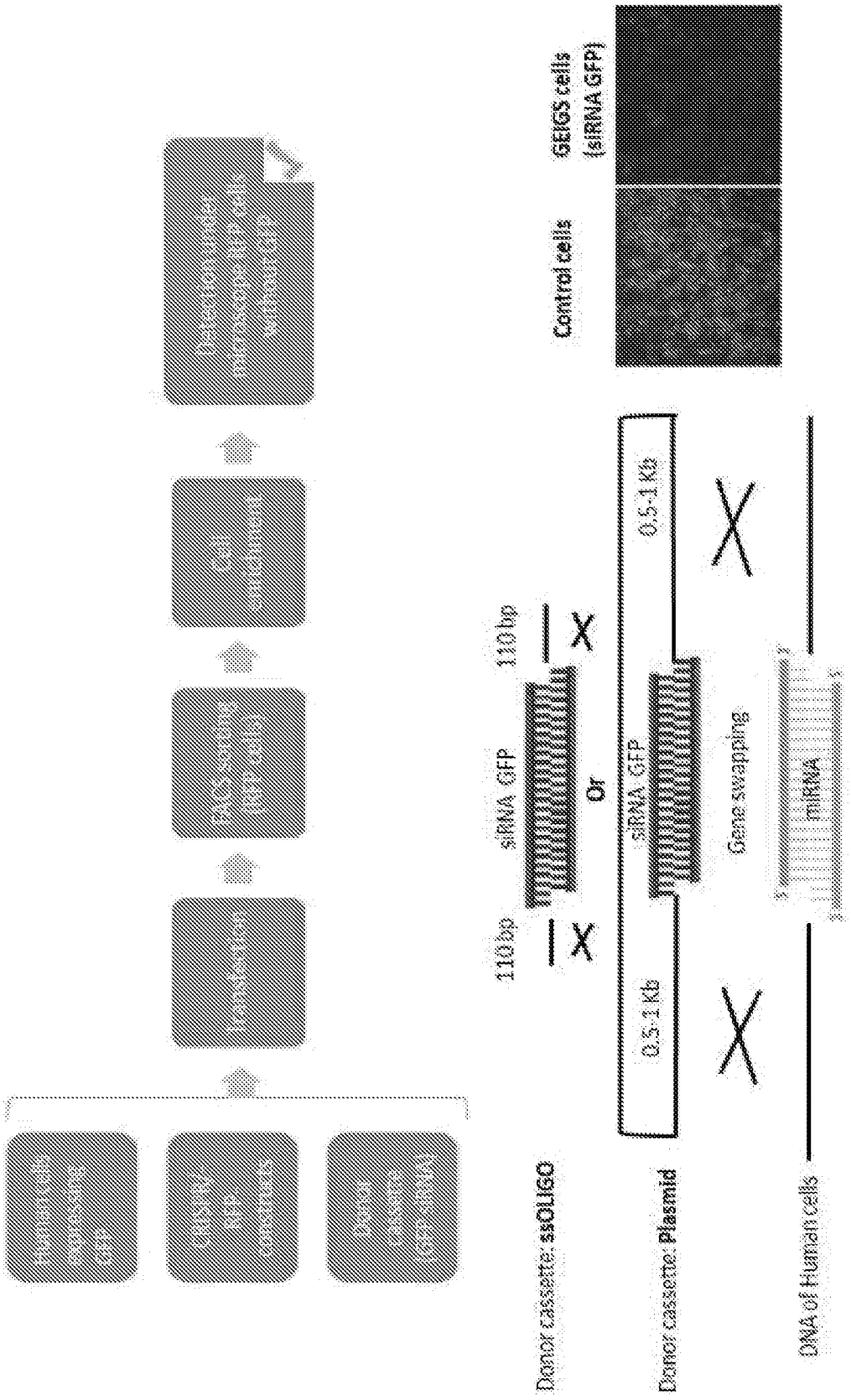

Control cells
GEIGS cells (siRNA GFP)
110 bp
siRNA GFP
110 bp
Or
Donor cassette: ssOLIGO
0.5-1 Kb
siRNA GFP
0.5-1 Kb
Donor cassette: Plasmid
Gene swapping
miRNA
DNA of Human cells

FIG. 3B



FIG. 3A

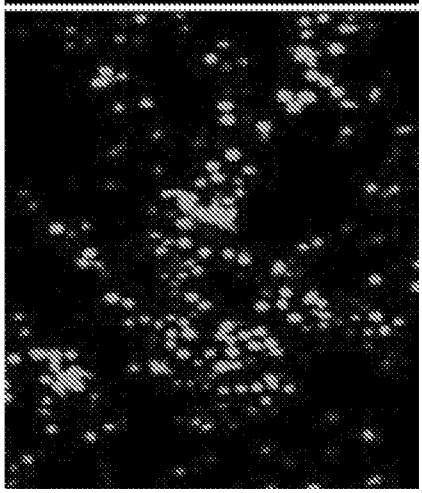


FIG. 4

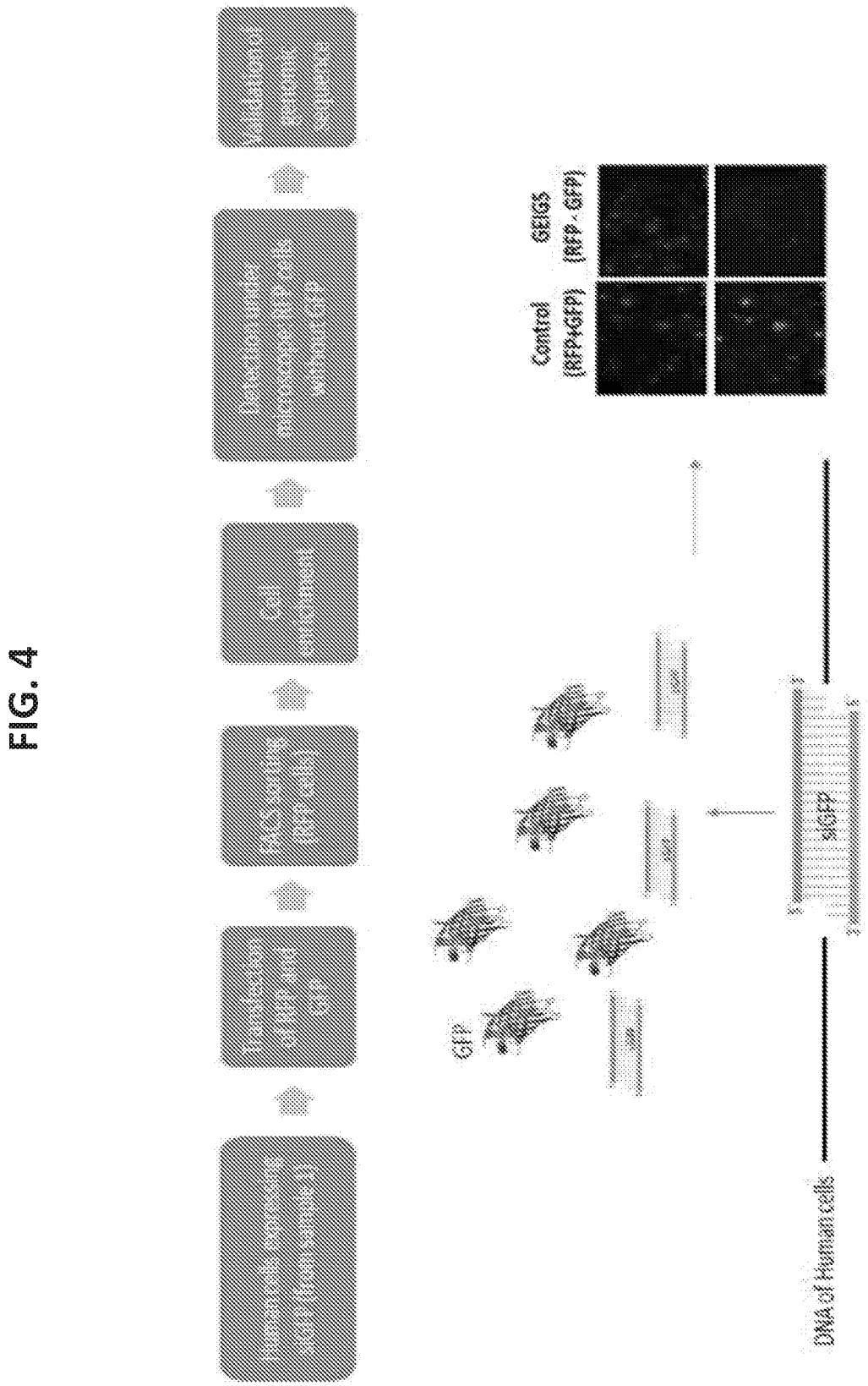


FIG. 5

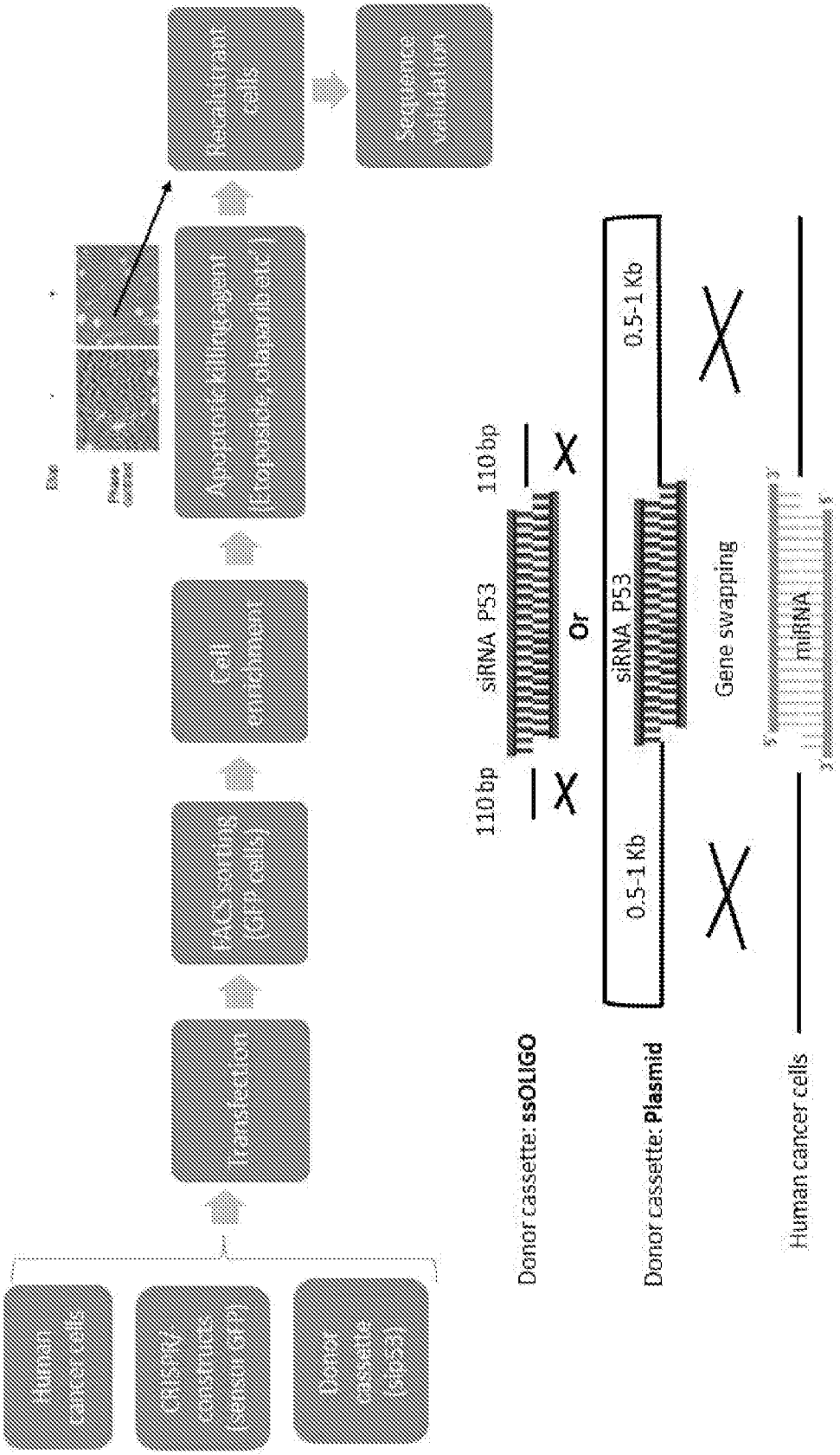

Human cancer cells
Donor cassette (siRNA)
Transfection
Cell recovery
Agarose entrapment (Hydrogel polymerization)
Cell recovery
Agarose entrapment (Hydrogel polymerization)
Transfection
Human cancer cells
Donor cassette: ssOLIGO
110 bp
siRNA P53
110 bp
Or
Donor cassette: Plasmid
0.5-1 Kb
siRNA P53
0.5-1 Kb
Gene swapping
Human cancer cells
miRNA

FIG. 6

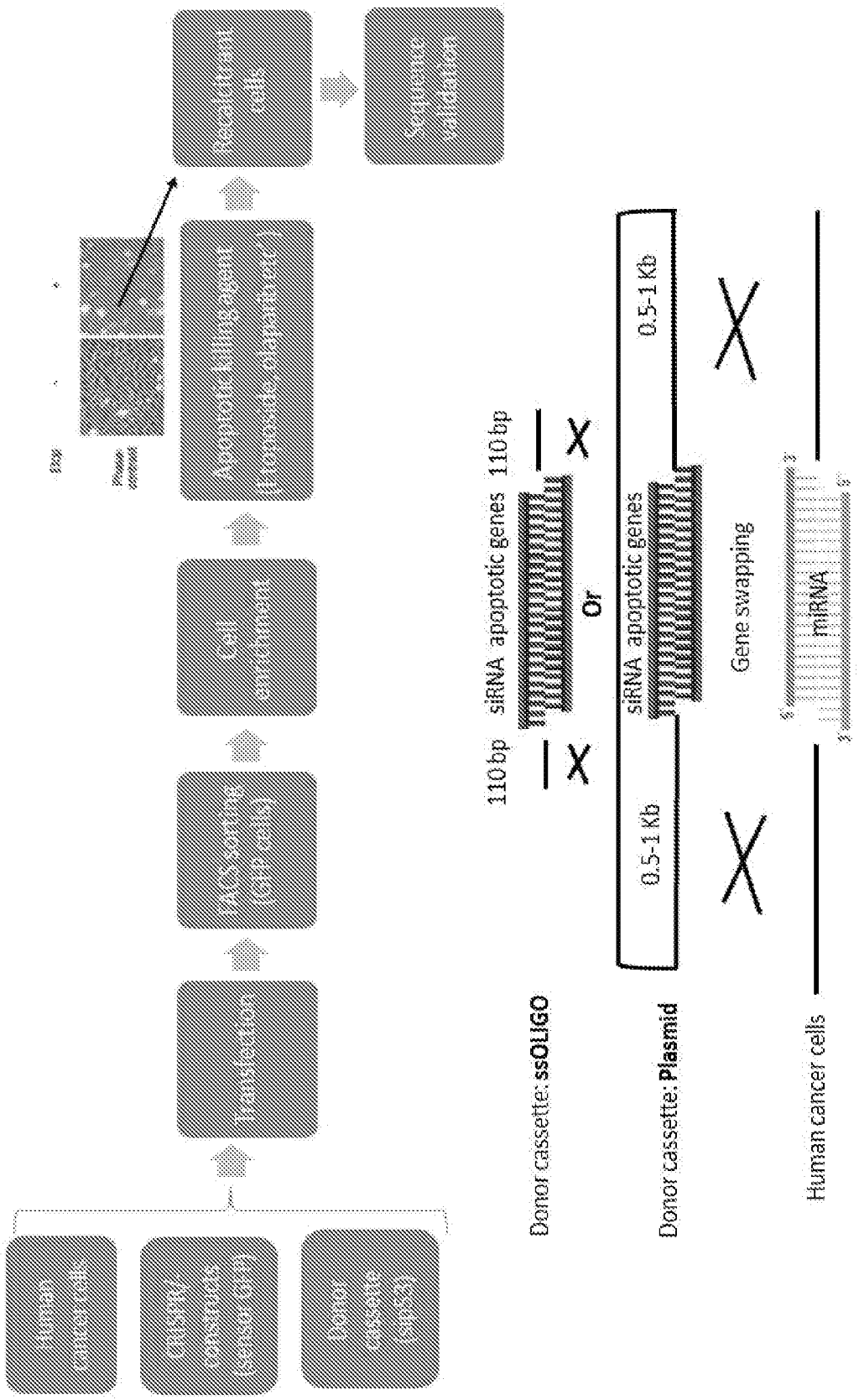

Human cancer cells
Donor cassette (ssOLIGO)
Donor cassette (Plasmid)
Transfection
Cell enrichment
Apoptotic killing assay
Recombinant cells
Sequencing validation
110 bp
siRNA apoptotic genes
110 bp
Or
0.5-1 Kb
siRNA apoptotic genes
0.5-1 Kb
Gene swapping
miRNA
Human cancer cells

FIG. 7

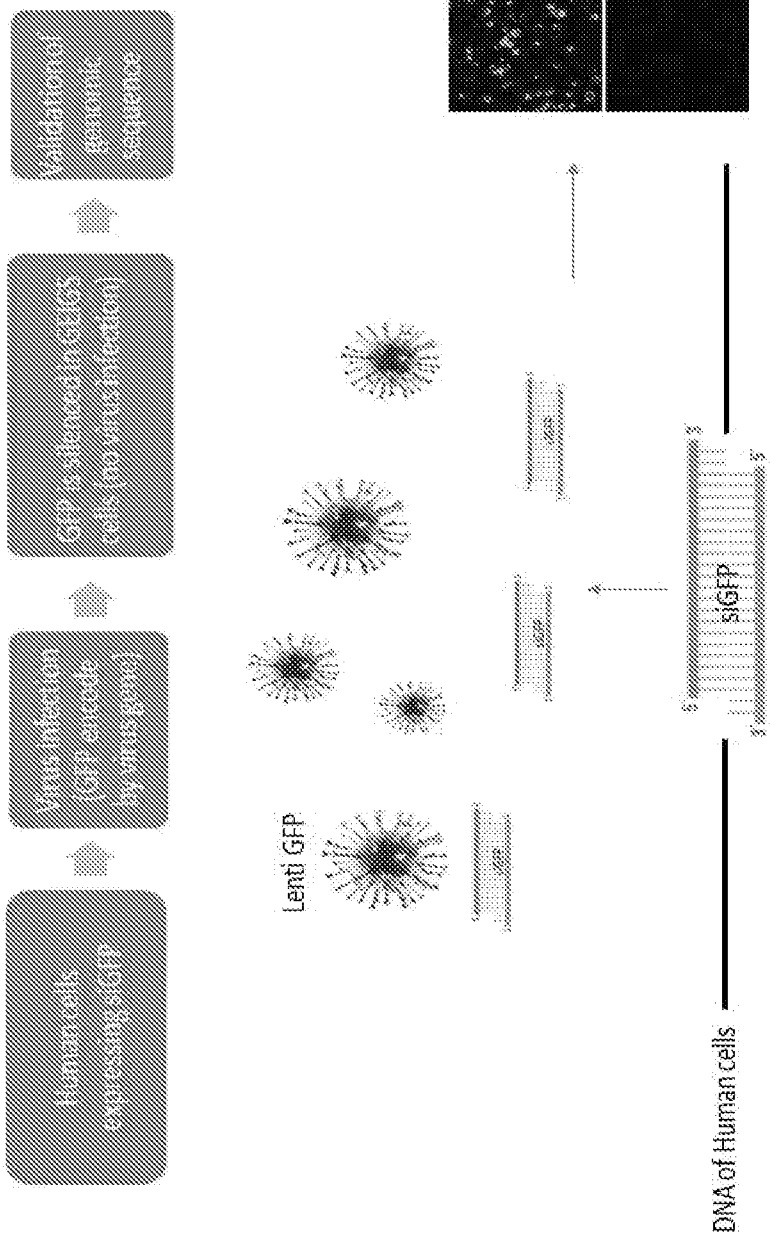

Human cells expressing no GFP
Viral infection (GFP encode lentiviruses)
GFP-expressing cells selection/amplification
Validation of genome editing
Lenti GFP
siGFP
DNA of Human cells
Control cells
Cells expressing siRNA against GFP (GFP silencing)

FIG. 8

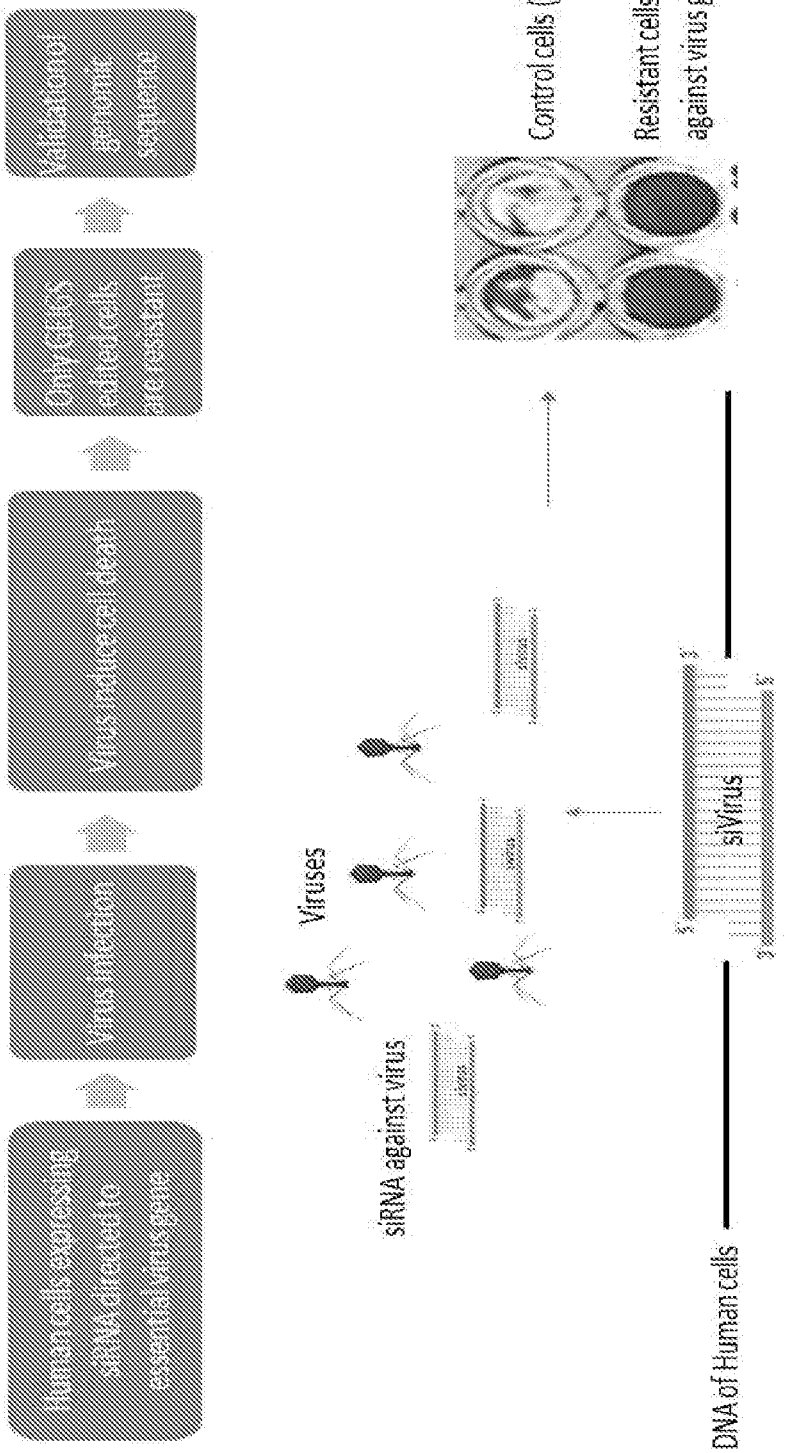

Human cells expressing siRNA directed to virus genome
Virus infection
Virus replication death
Control cells (less viable cells)
Resistant cells expressing siRNA against virus genes
Viruses
siRNA against virus
siVirus
DNA of Human cells

FIG. 9

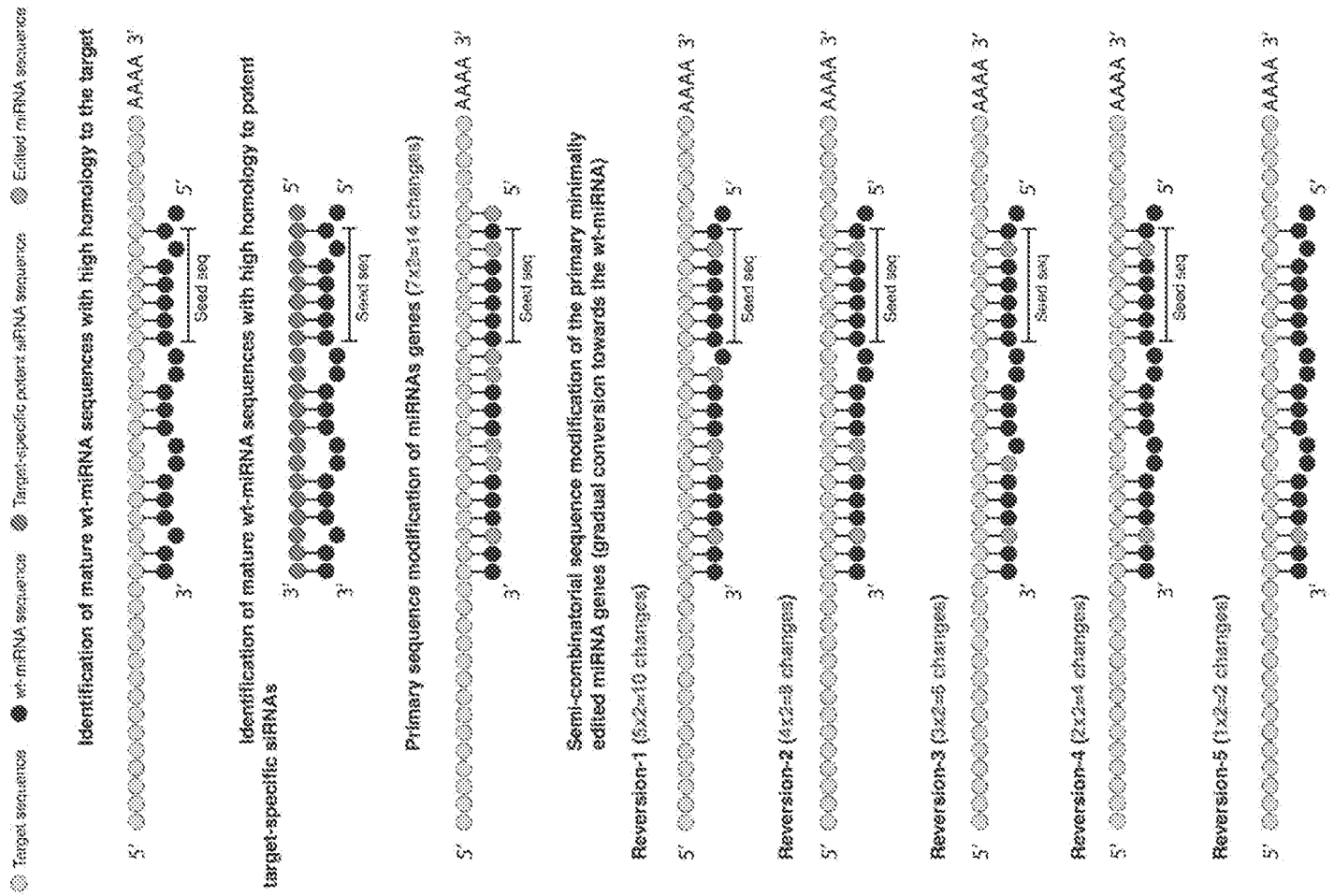
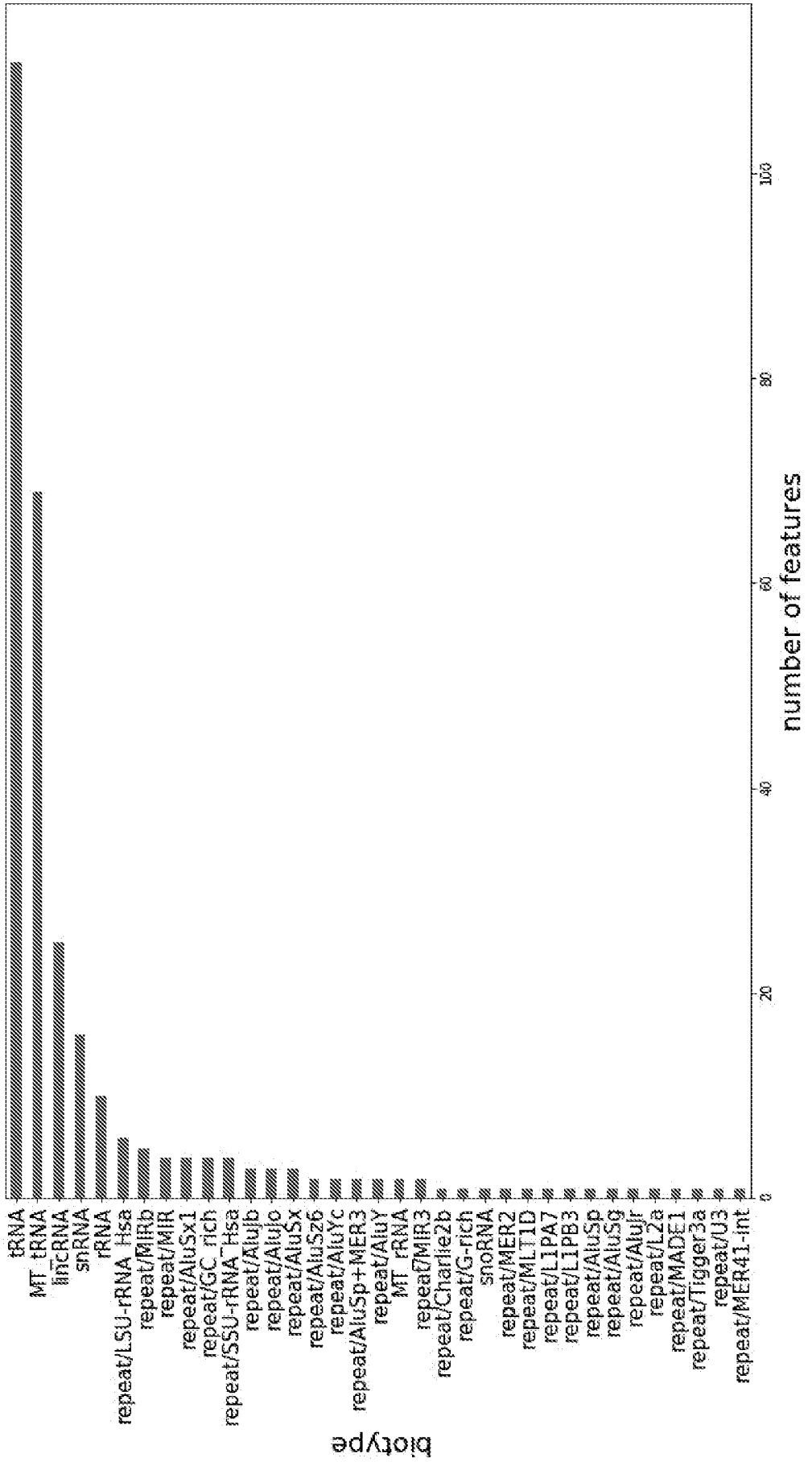

Target sequence
wt-miRNA sequence
Target-specific potent siRNA sequence
Edited miRNA sequence
Identification of mature wt-miRNA sequences with high homology to the target
5'
3'
AAAA 3'
5'
Seed seq
Identification of mature wt-miRNA sequences with high homology to potent target-specific siRNAs
3'
5'
3'
5'
Seed seq
Primary sequence modification of miRNAs genes (7x2=14 changes)
5'
3'
AAAA 3'
5'
Seed seq
Semi-combinatorial sequence modification of the primary minimally edited miRNA genes (gradual conversion towards the wt-miRNA)
Reversion-1 (6x2=10 changes)
5'
3'
AAAA 3'
5'
Seed seq
Reversion-2 (4x2=8 changes)
5'
3'
AAAA 3'
5'
Seed seq
Reversion-3 (3x2=6 changes)
5'
3'
AAAA 3'
5'
Seed seq
Reversion-4 (2x2=4 changes)
5'
3'
AAAA 3'
5'
Seed seq
Reversion-5 (1x2=2 changes)
5'
3'
AAAA 3'
5'

FIG. 10


tRNA
MT_tRNA
lincRNA
snRNA
rRNA
repeat/LSU-rRNA_Hsa
repeat/MIRb
repeat/MIR
repeat/AluSx1
repeat/GC_rich
repeat/SSU-rRNA_Hsa
repeat/AluJb
repeat/AluJo
repeat/AluSx
repeat/AluS26
repeat/AluYc
repeat/AluSp+MER3
repeat/AluY
MT_rRNA
repeat/MIR3
repeat/Charlie2b
repeat/G-rich
snoRNA
repeat/MER2
repeat/MLT1D
repeat/L1PA7
repeat/L1PB3
repeat/AluSp
repeat/AluSg
repeat/AluJr
repeat/L2a
repeat/MADE1
repeat/Tigger3a
repeat/U3
repeat/MER41-int
biotype
0
20
40
60
80
100
number of features

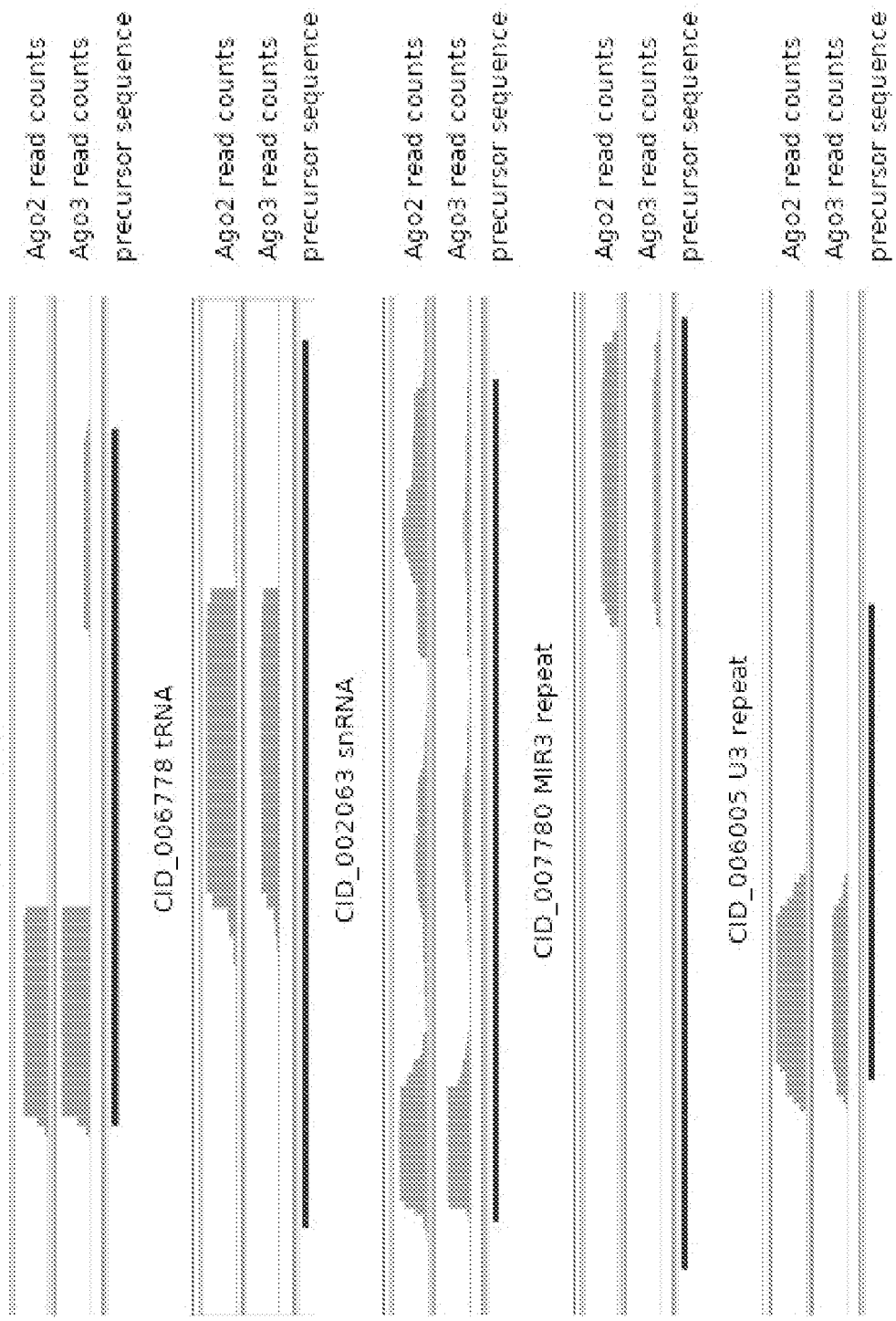


FIG. 11A

FIG. 11B

FIG. 11C

FIG. 11D

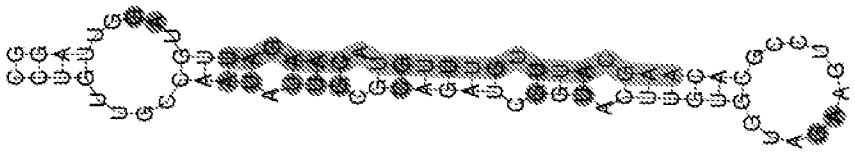
FIG. 11E

FIG. 12A



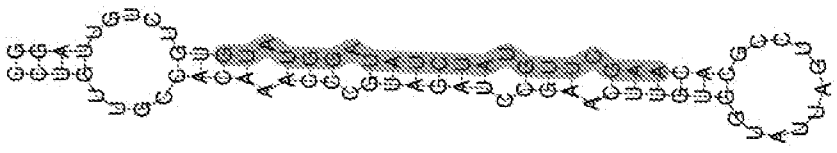
GEiGS-based on miR100 (large nt change)

SEQ ID NO: 255



GEiGS-based on miR100 (small nt change)

SEQ ID NO: 254



Wild-type miR100

SEQ ID NO: 253

FIG. 12B

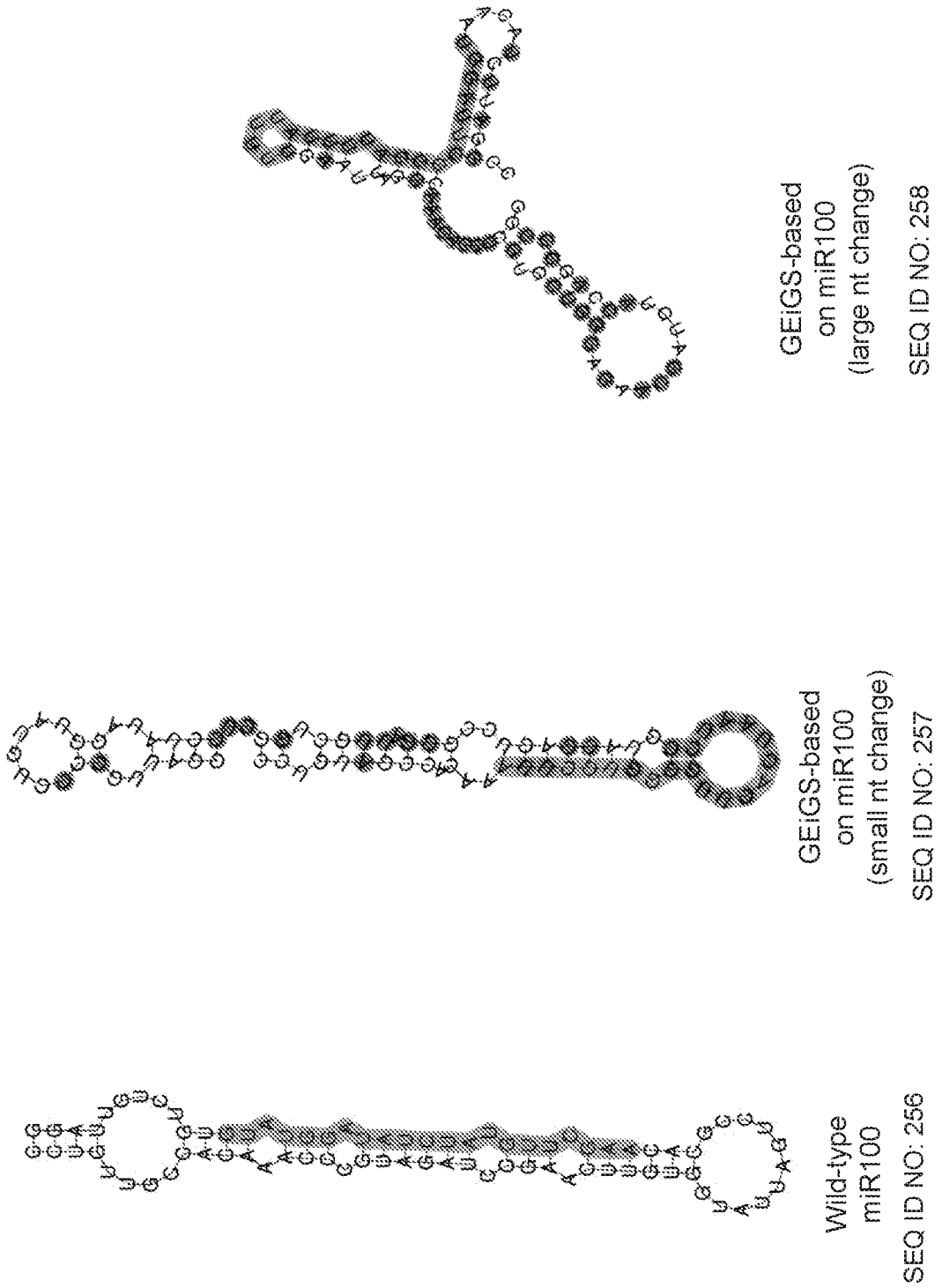
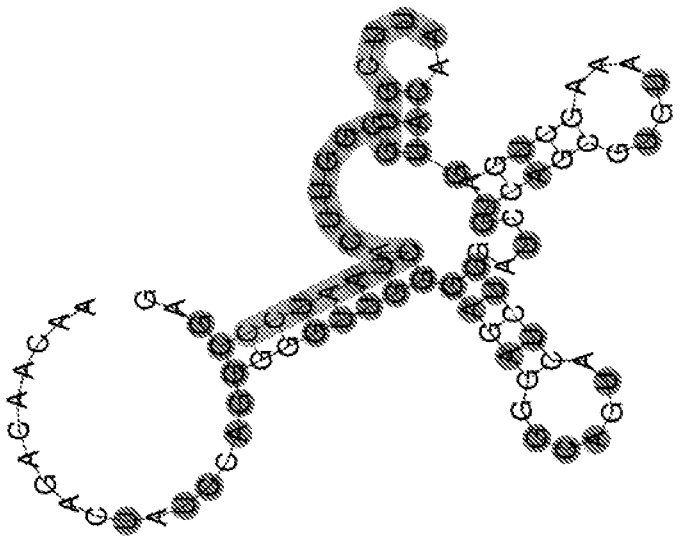
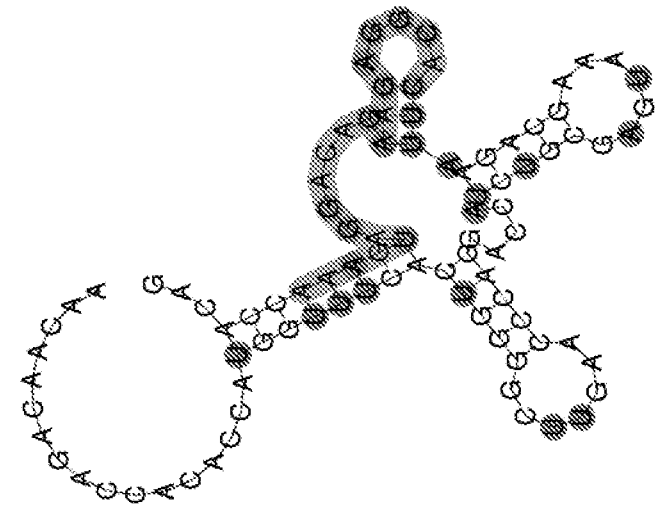

Wild-type miR100
SEQ ID NO: 256
GEiGS-based on miR100 (small nt change)
SEQ ID NO: 257
GEiGS-based on miR100 (large nt change)
SEQ ID NO: 258

FIG. 12C



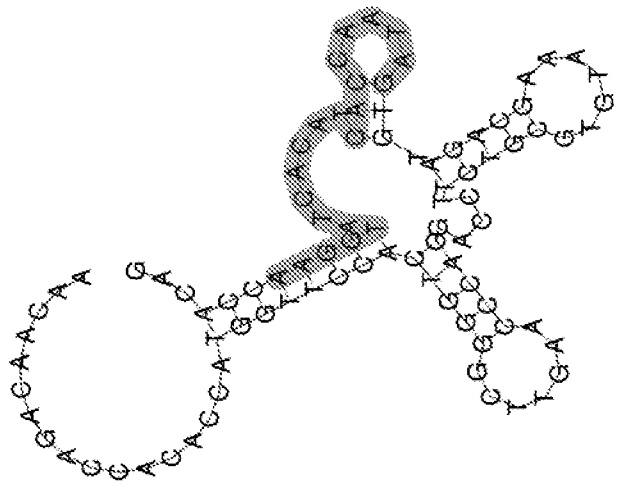
GEiGS-based on tRNA (large nt change)

SEQ ID NO: 261



GEiGS-based on tRNA (small nt change)

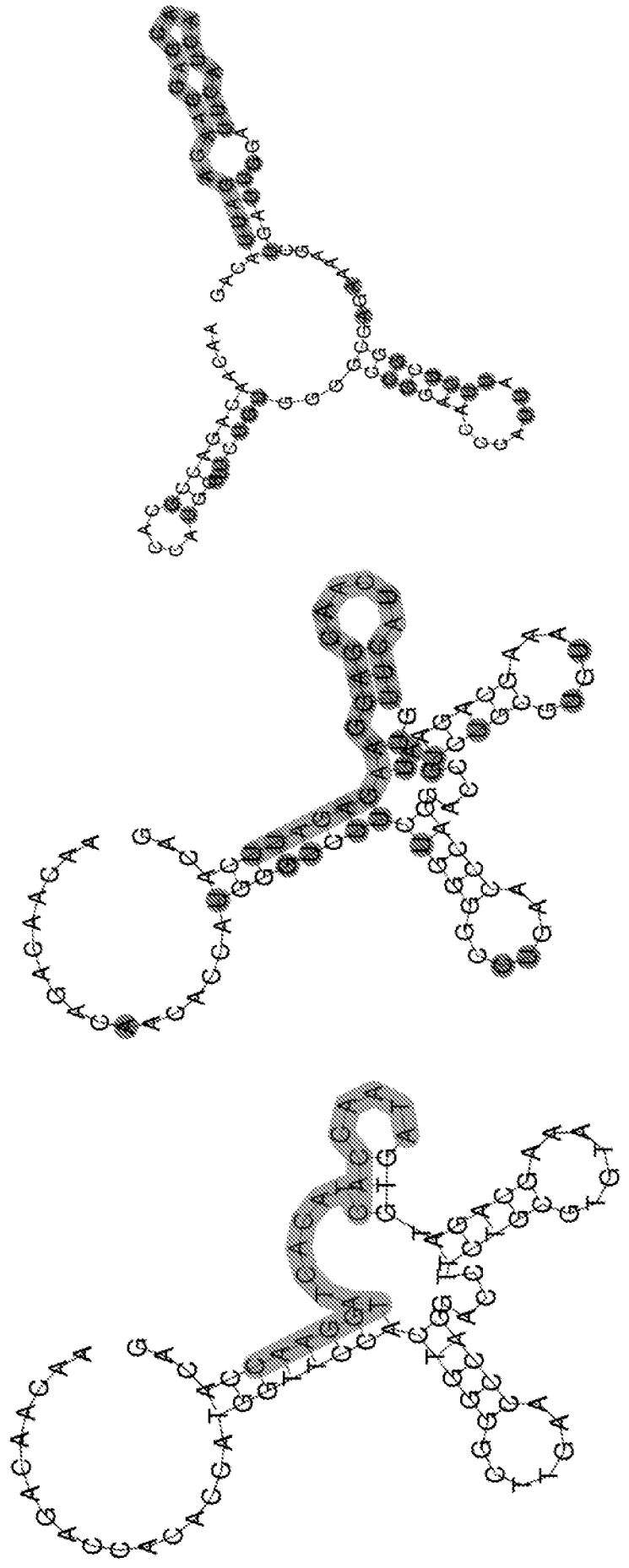
SEQ ID NO: 260



Wild-type tRNA

SEQ ID NO: 259

FIG. 12D



Wild-type tRNA

SEQ ID NO: 262

GEiGS-based on tRNA (small nt change)

SEQ ID NO: 263

GEiGS-based on tRNA (large nt change)

SEQ ID NO: 264

FIG. 12E

SEQ ID NO: 265
Wild-type
tasiRNA precursor
SEQ ID NO: 266
GEiGS-based
on tasiRNA precursor
(small nt change)
SEQ ID NO: 267
GEiGS-based
on tasiRNA precursor
(large nt change)

FIG. 13

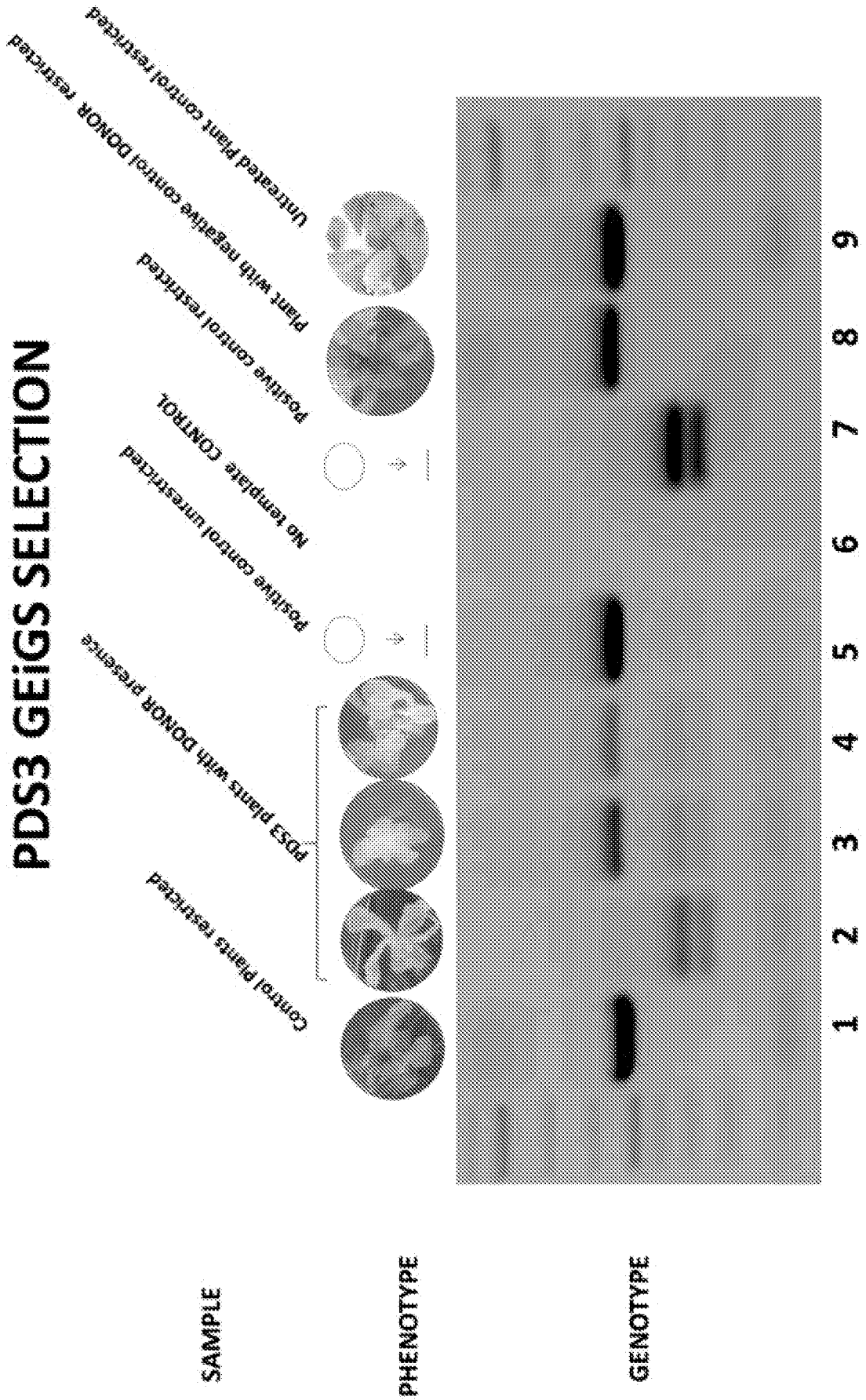

PDS3 GEiGS SELECTION
SAMPLE
PHENOTYPE
GENOTYPE
Control plants restricted
PDS3 plants with DONOR presence
Positive control unrestricted
No template CONTROL
Positive control restricted
Plant with negative control DONOR restricted
Untreated plant control restricted
1 2 3 4 5 6 7 8 9

FIG. 14

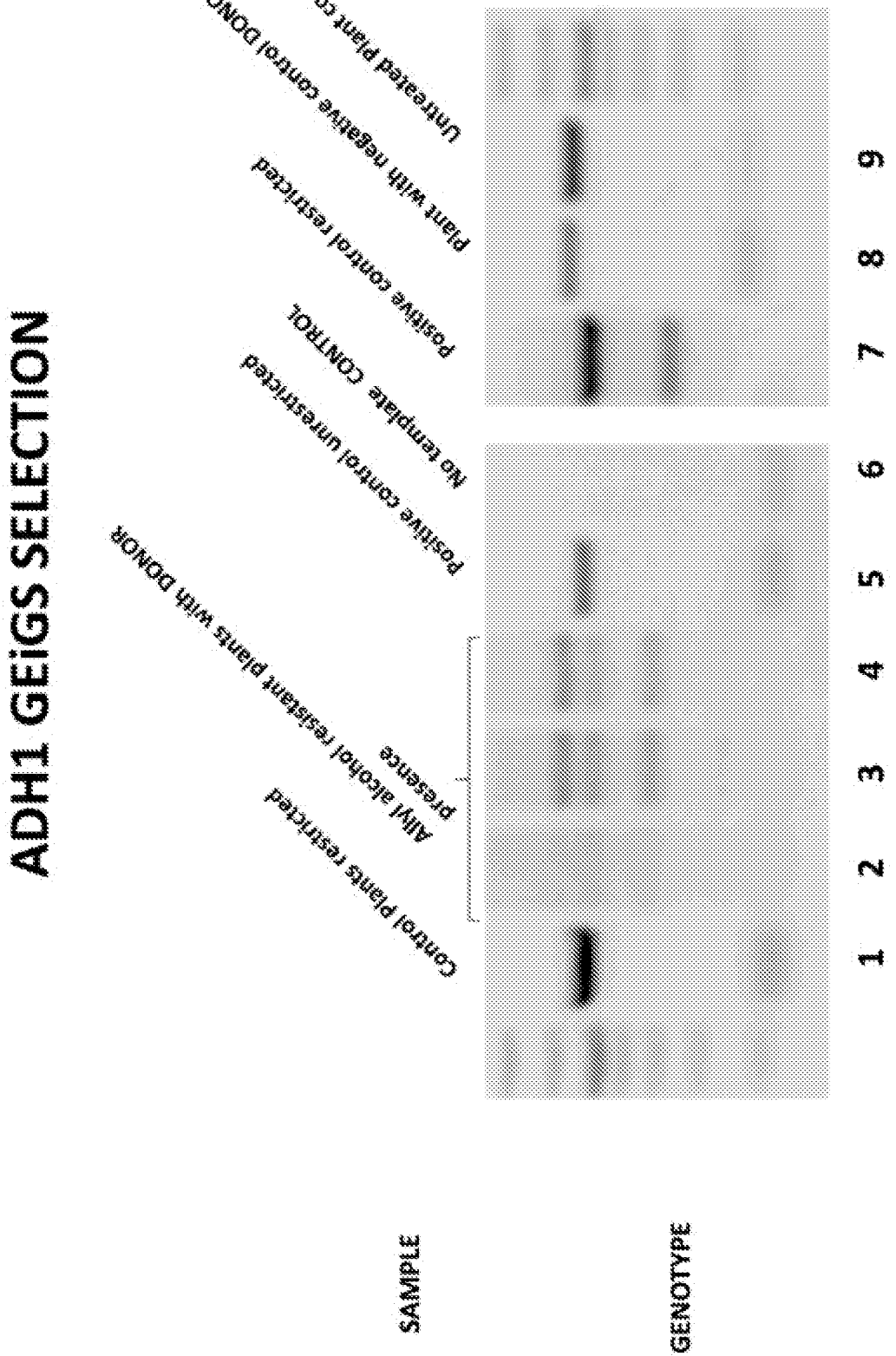

ADH1 GEiGS SELECTION
SAMPLE
GENOTYPE
1 2 3 4 5 6 7 8 9
Control plants resistant
AlkV alcohol resistant plants with DONOR
presence
Positive control unresistant
No template CONTROL
Positive control resistant
Plant with negative control DONOR resistant
Untreated plant control resistant

FIG. 15

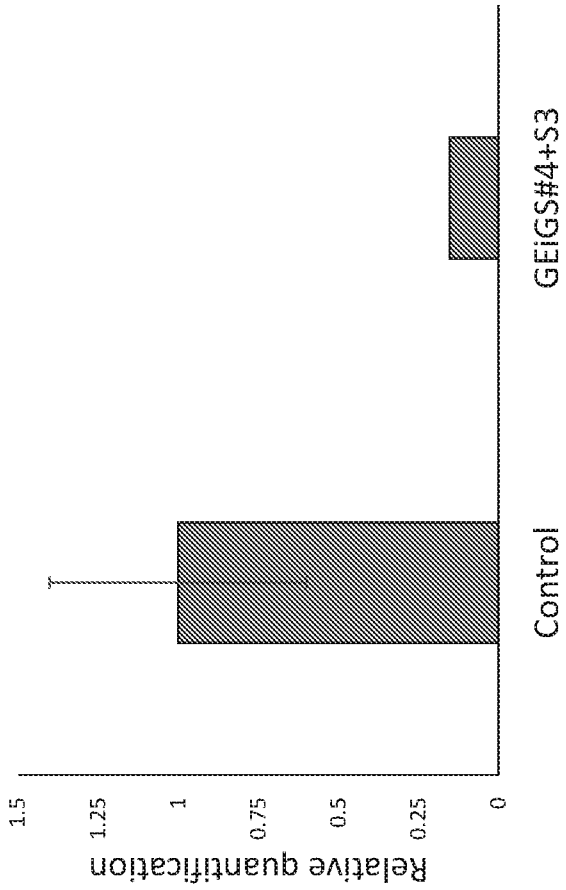
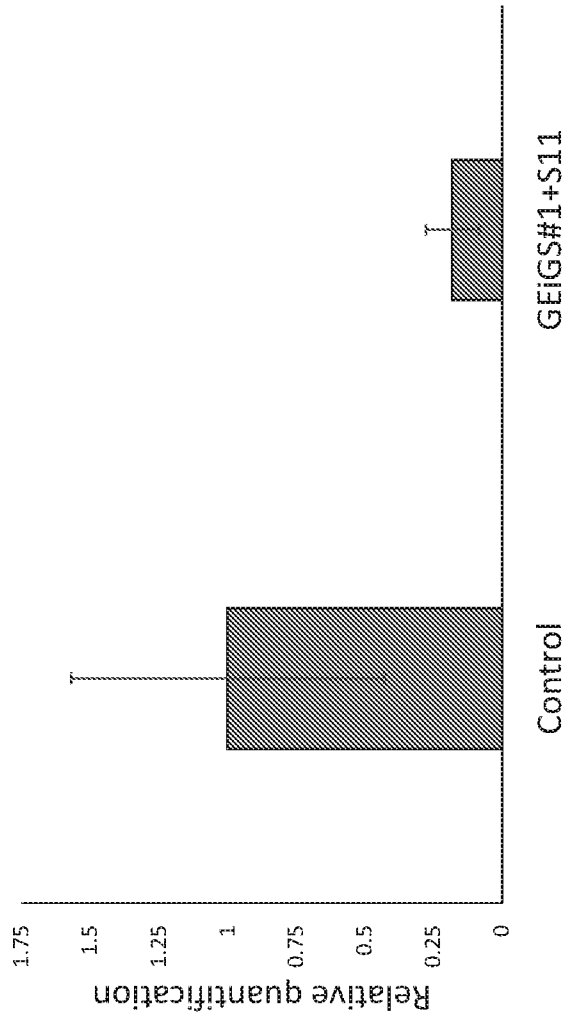

AtPDS3 expression in GEiGS#4+SWAP3 modified plant
Relative quantification
1.5
1.25
1
0.75
0.5
0.25
0
Control
GEiGS#4+S3

FIG. 16


AtADH1 expression in GEiGS#1+SWAP11 modified plant
Relative quantification
1.75
1.5
1.25
1
0.75
0.5
0.25
0
Control
GEiGS#1+S11