FIG. 1

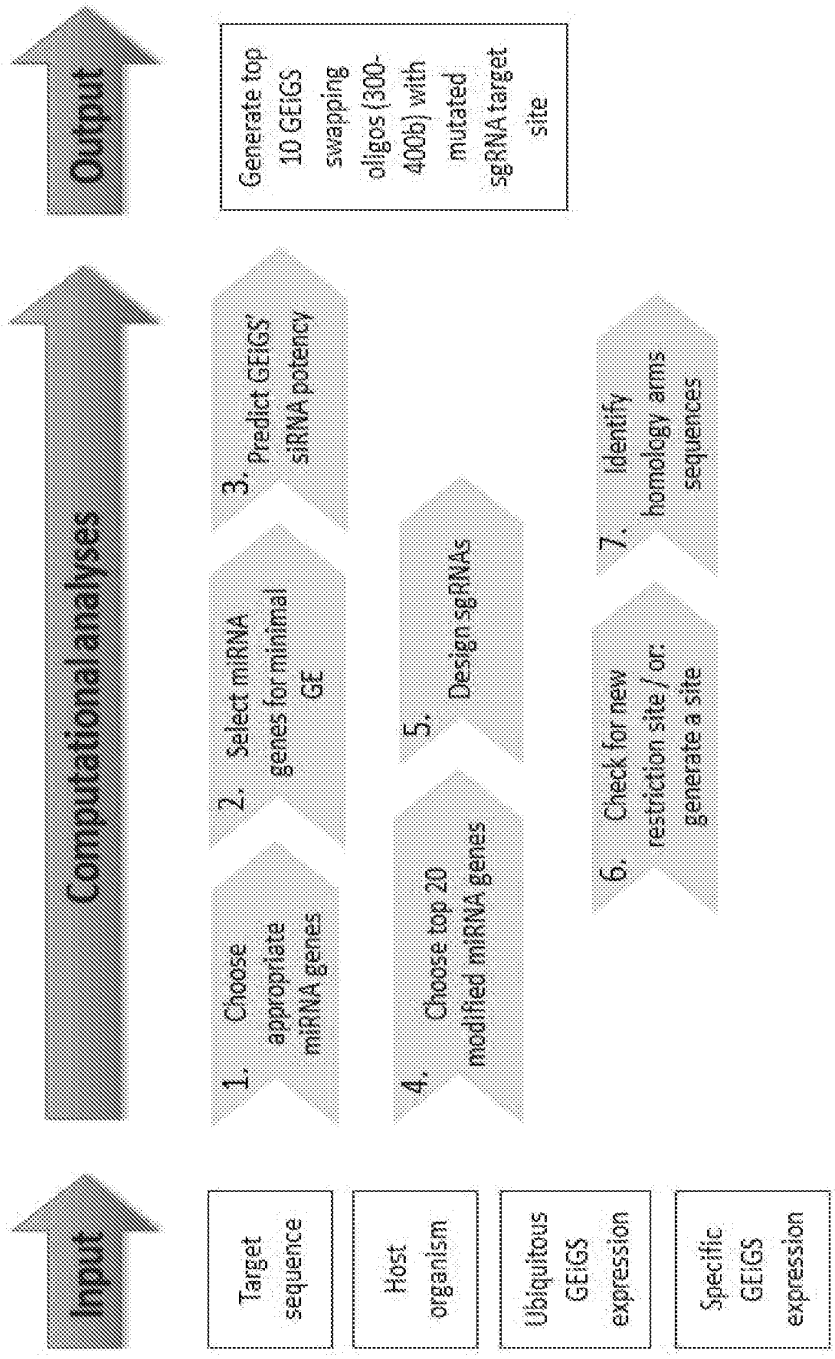

Input
Target sequence
Host organism
Ubiquitous GEIGS expression
Specific GEIGS expression
Computational analyses
1. Choose appropriate miRNA genes
2. Select miRNA genes for minimal GE
3. Predict GEIGS' siRNA potency
4. Choose top 20 modified miRNA genes
5. Design sgRNAs
6. Check for new restriction site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

FIG. 2

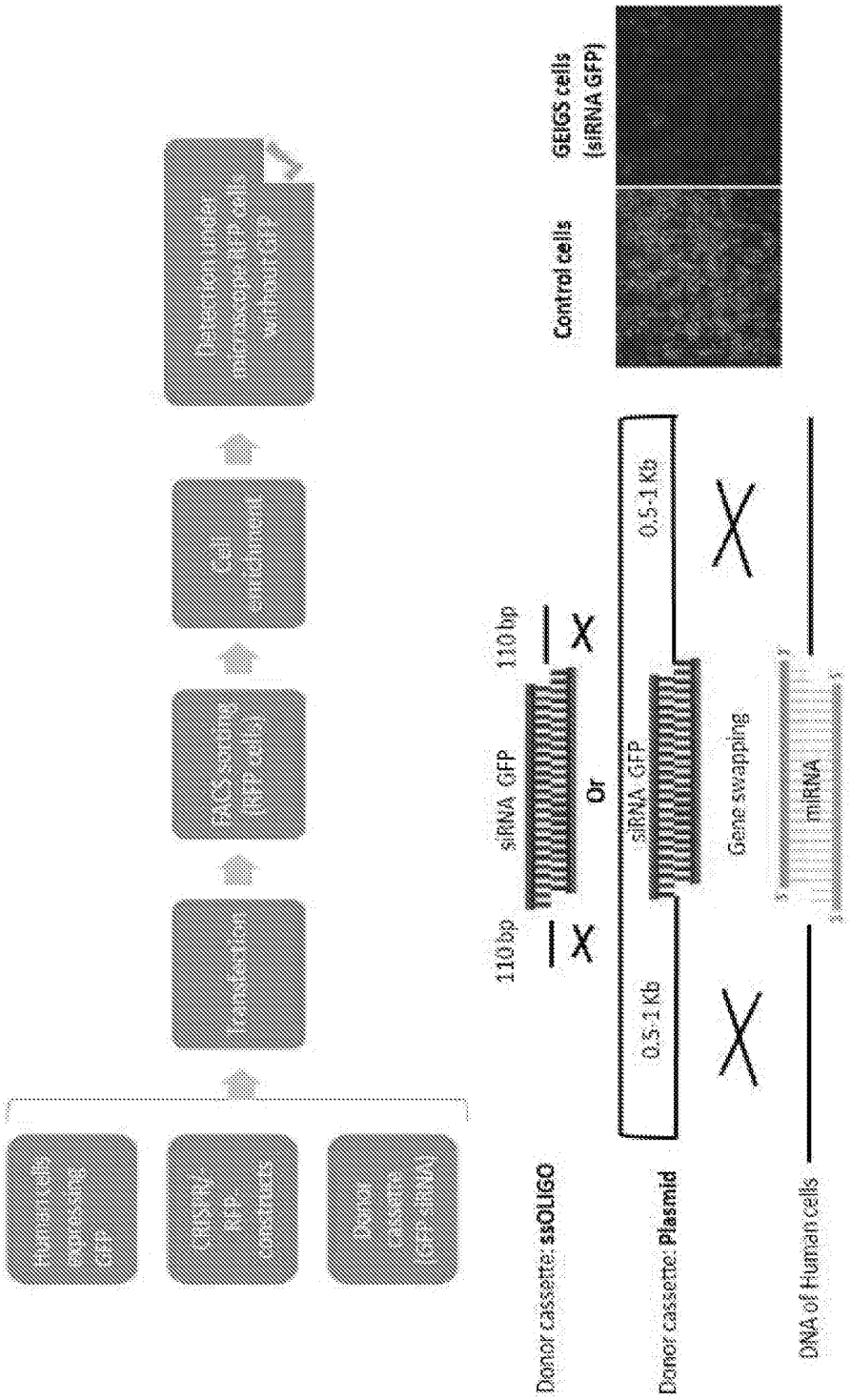

Control cells
GEIGS cells (siRNA GFP)
Donor cassette: ssOLIGO
110 bp
siRNA GFP
110 bp
Or
Donor cassette: Plasmid
0.5-1 Kb
siRNA GFP
0.5-1 Kb
Gene swapping
DNA of Human cells
miRNA

FIG. 3B



FIG. 3A

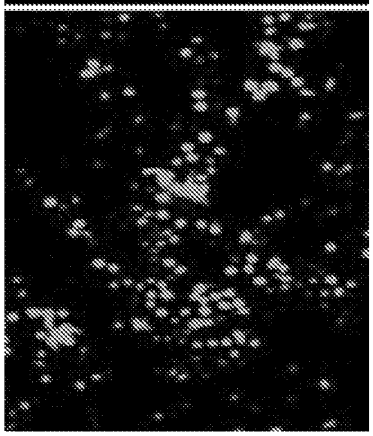


FIG. 4

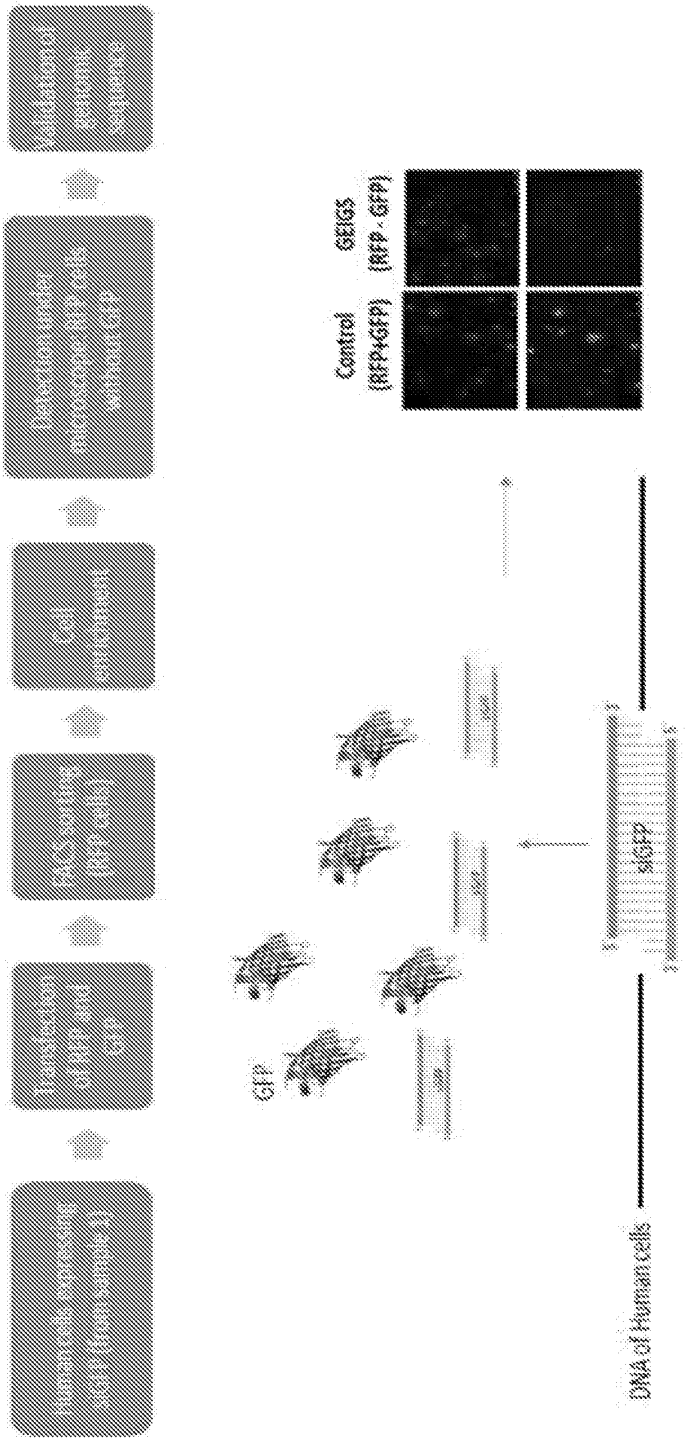


FIG. 5

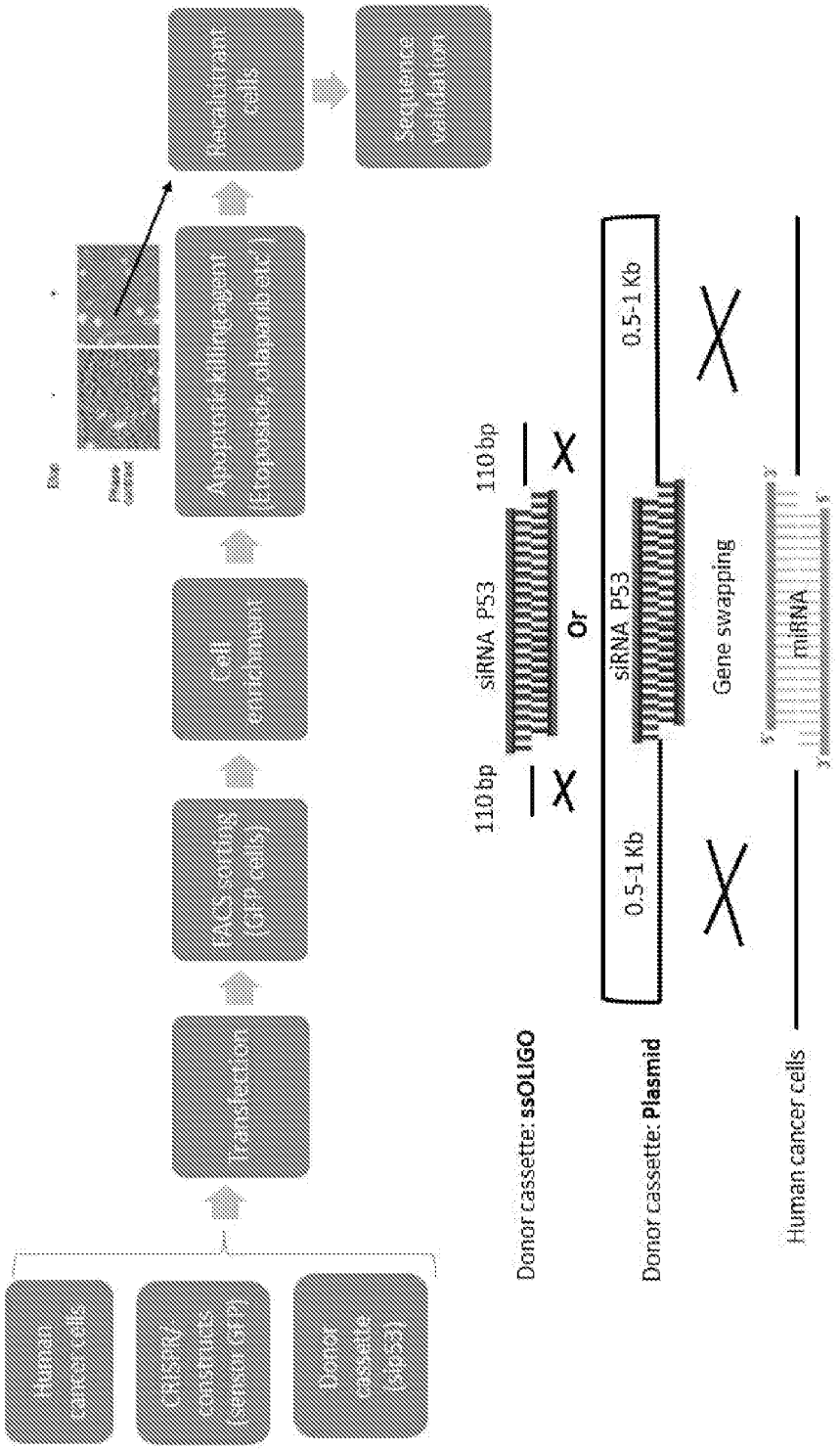

Human cancer cells
Cas9/gRNA complexes (ribonucleoprotein)
Donor cassette (plasmid)
Transfection
FACS sorting (GFP+ cells)
Cell enrichment
Apoptotic differentiation (caspase-mediated)
Blue
Orange
Red
Recombinant cells
Sequence validation
Donor cassette: ssOLIGO
110 bp
siRNA P53
110 bp
Or
Donor cassette: Plasmid
0.5-1 Kb
siRNA P53
0.5-1 Kb
Gene swapping
Human cancer cells
miRNA

FIG. 6

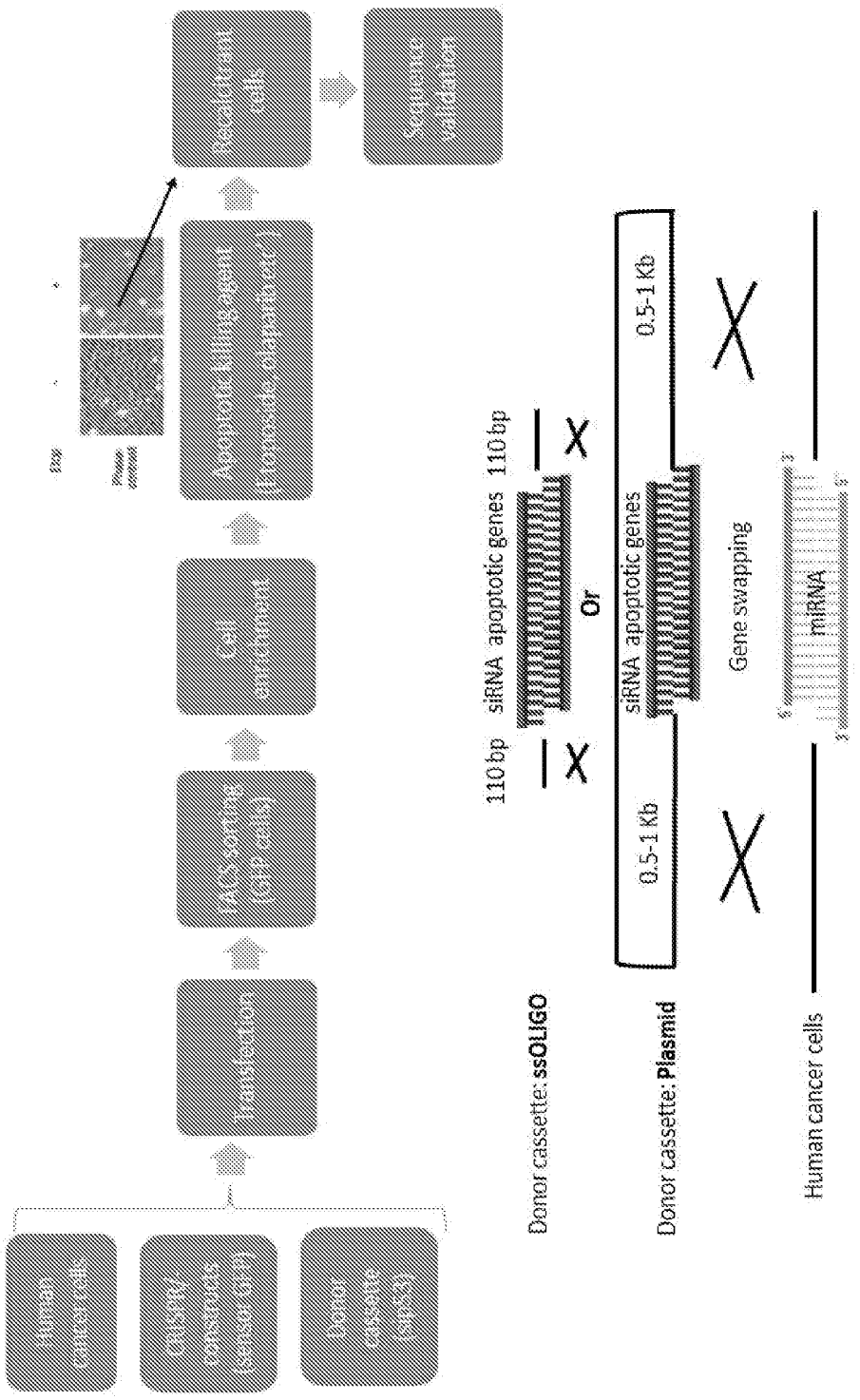

Human cancer cells
Colony forming assay (CFA)
Donor cassette (siRNA)
Transduction
HACS-based (GFP cells)
Cell enrichment
Apoptosis-killing agent (Caspase-dependent)
Recalcitrant cells
Sequence validation
Human cancer cells
Donor cassette: ssOLIGO
110 bp
siRNA apoptotic genes
110 bp
Or
Donor cassette: Plasmid
0.5-1 Kb
siRNA apoptotic genes
0.5-1 Kb
Gene swapping
miRNA

FIG. 7

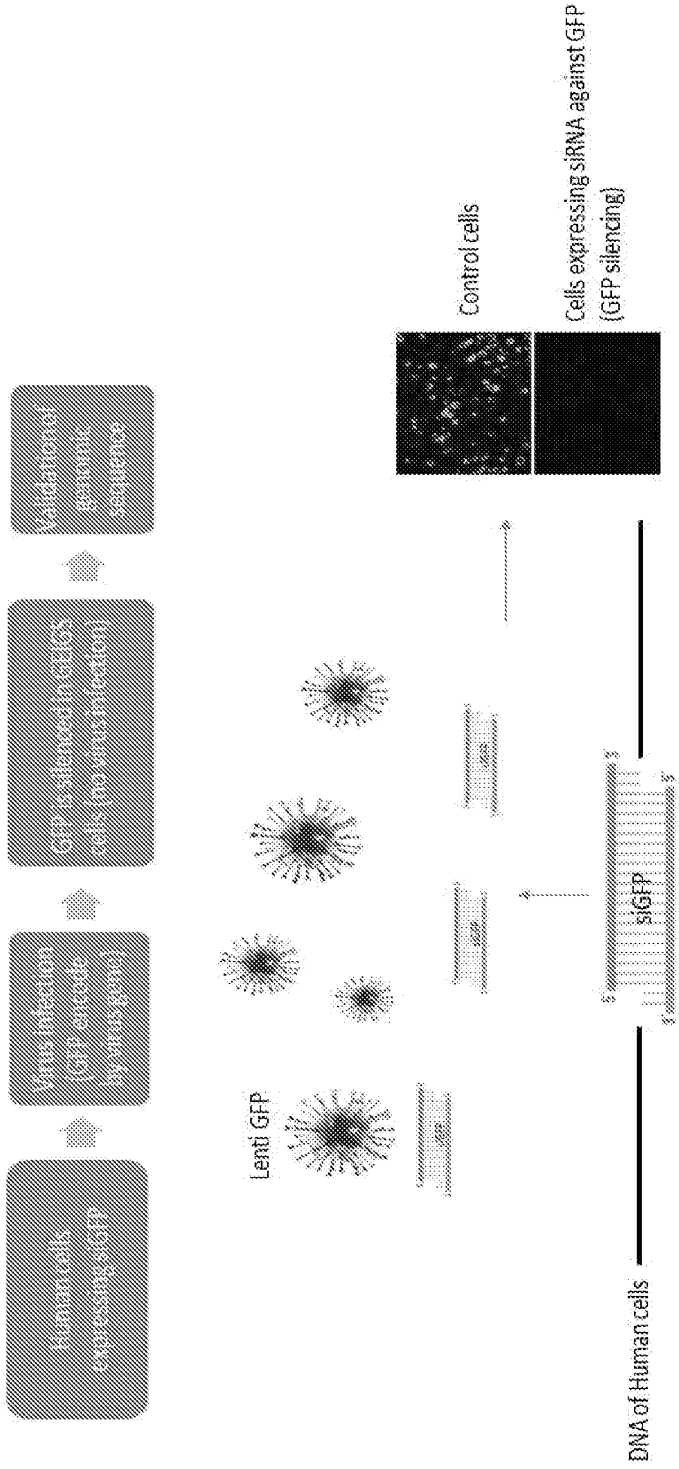

Human cells expressing GFP
Viral infection (lentiviral vector)
Gene silencing (GFP) cells (transduction)
Validation of genome sequence
Lenti GFP
DNA of Human cells
siGFP
Control cells
Cells expressing siRNA against GFP (GFP silencing)

FIG. 8

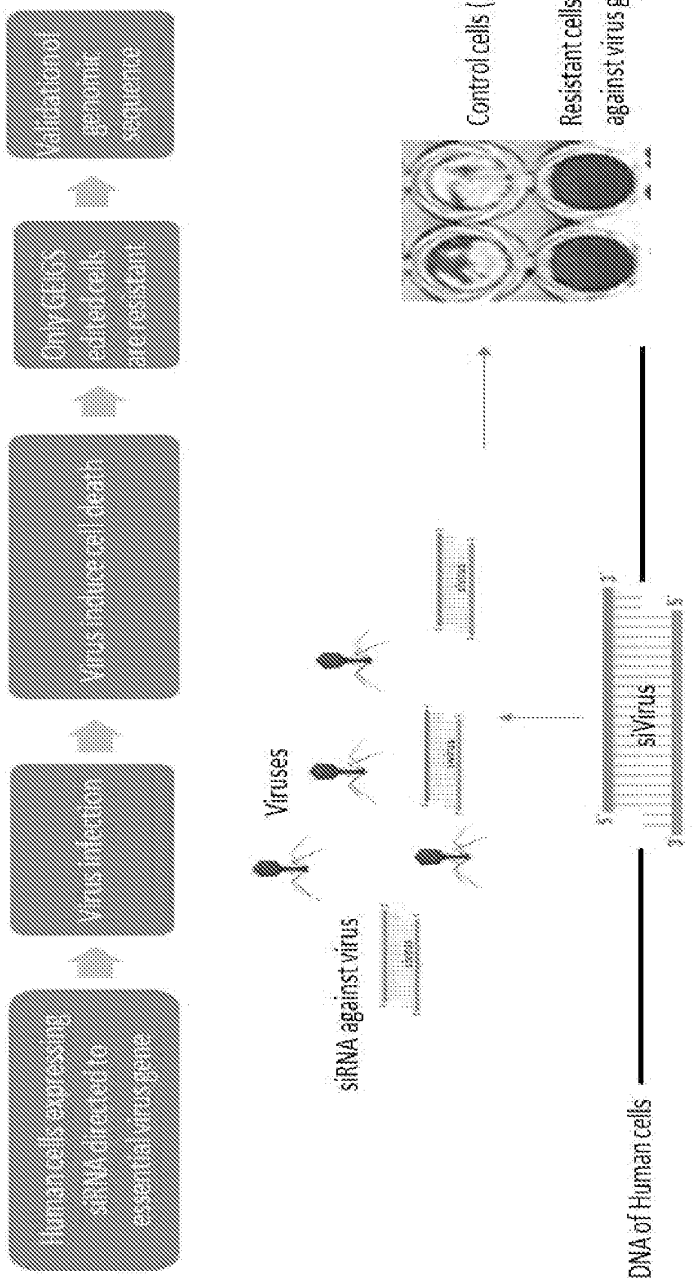

Human cells expressing siRNA against virus genes
Virus infection
Virus induces cell death
Only cells edited cells are resistant
Validation of genes sequence
siRNA against virus
Viruses
siVirus
DNA of Human cells
Control cells (less viable cells)
Resistant cells expressing siRNA against virus genes

FIG. 9

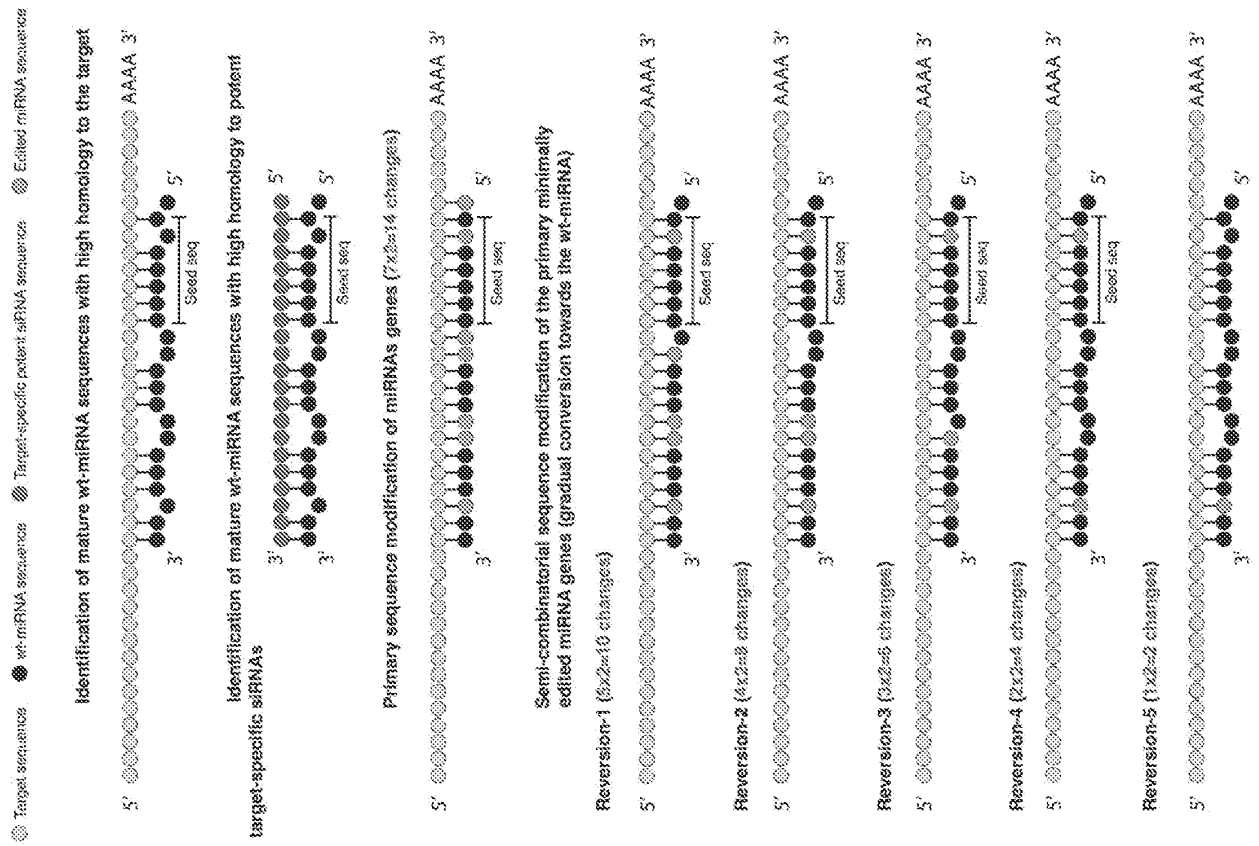
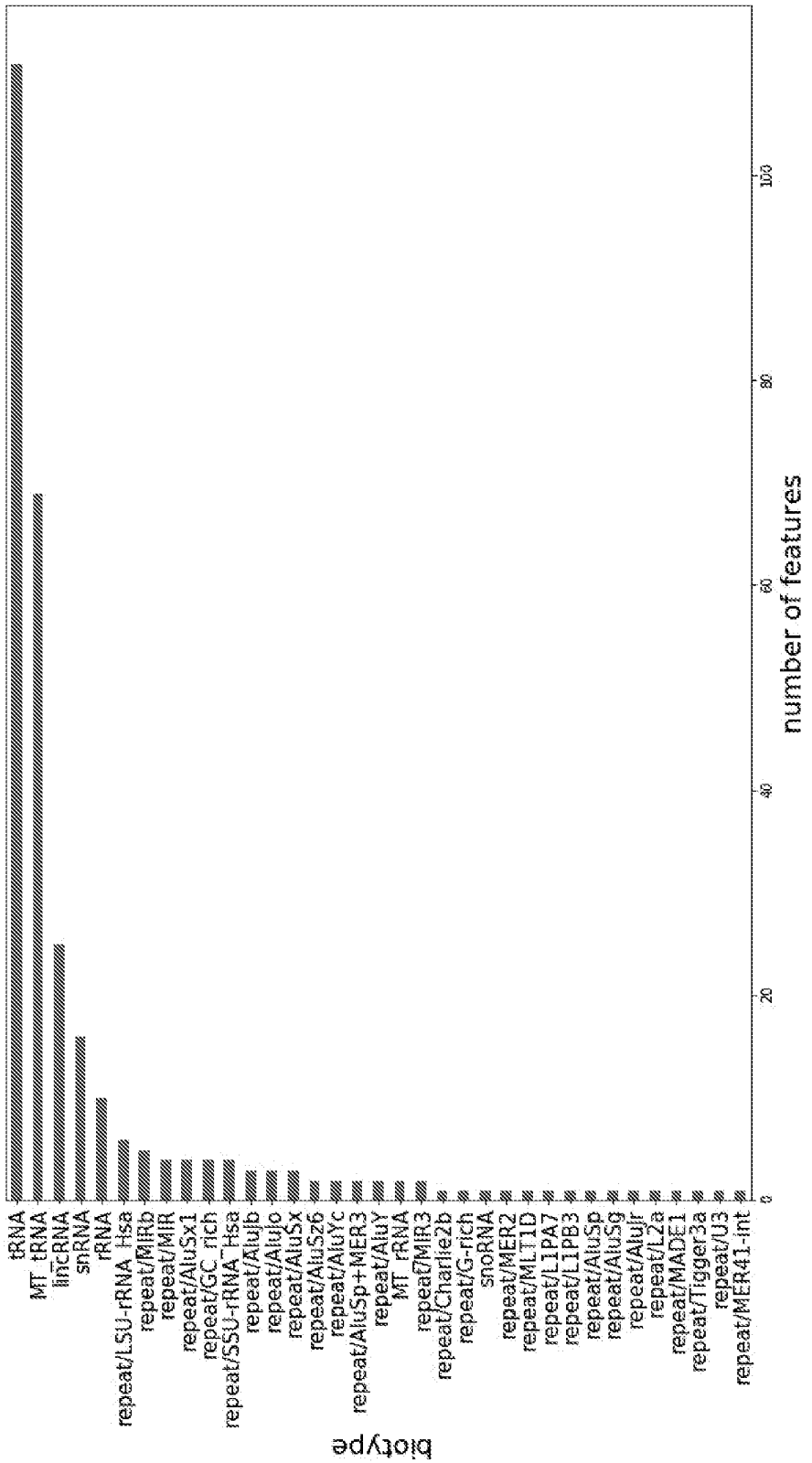

Target sequence
wt-miRNA sequence
Target-specific potent siRNA sequence
Edited miRNA sequence
Identification of mature wt-miRNA sequences with high homology to the target
5'
3'
AAAA 3'
Seed seq
Identification of mature wt-miRNA sequences with high homology to potent target-specific siRNAs
3'
5'
Primary sequence modification of miRNAs genes (7x2e14 changes)
Semi-combinatorial sequence modification of the primary minimally edited miRNA genes (gradual conversion towards the wt-miRNA)
Reversion-1 (6x2e10 changes)
Reversion-2 (4x2e8 changes)
Reversion-3 (3x2e6 changes)
Reversion-4 (2x2e4 changes)
Reversion-5 (1x2e2 changes)

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/AluSz6
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

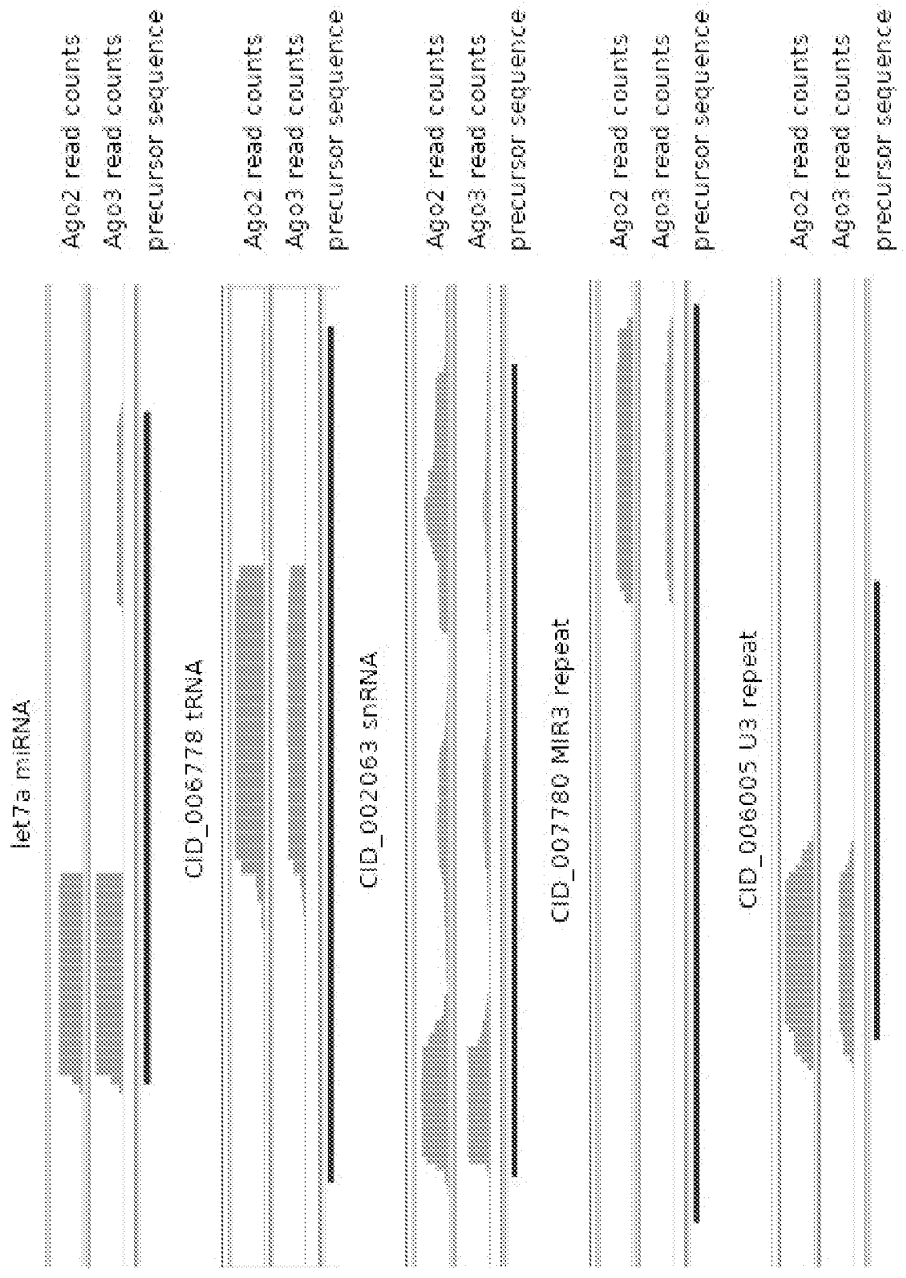


FIG. 11A

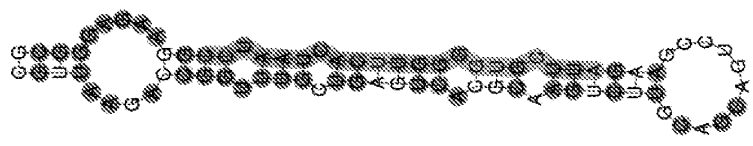
FIG. 11B

FIG. 11C

FIG. 11D

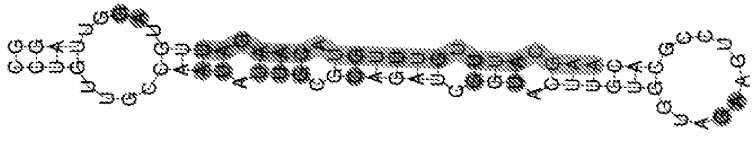
FIG. 11E

FIG. 12A



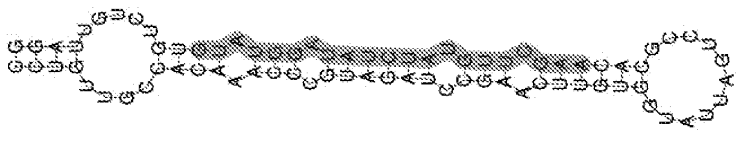
GEiGS-based on miR100 (large nt change)

SEQ ID NO: 255



GEiGS-based on miR100 (small nt change)

SEQ ID NO: 254



Wild-type miR100

SEQ ID NO: 253

FIG. 12B

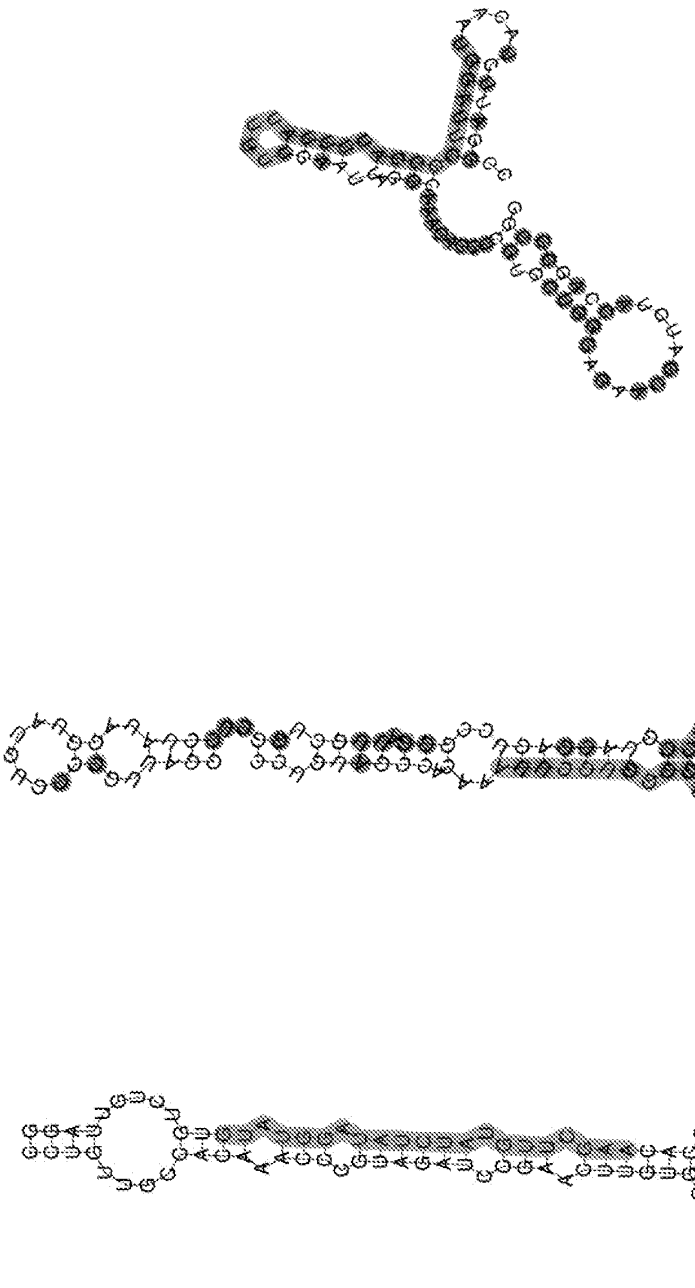
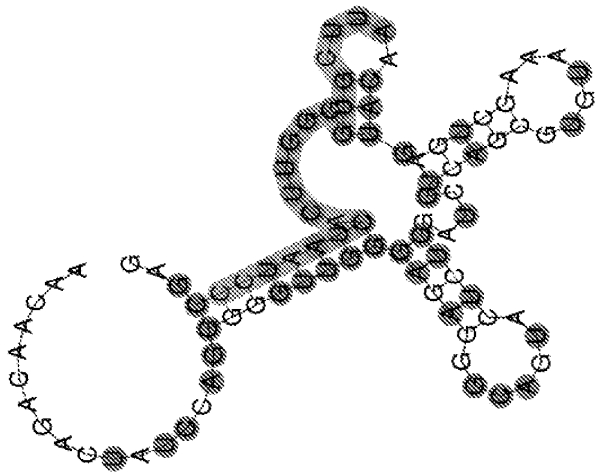
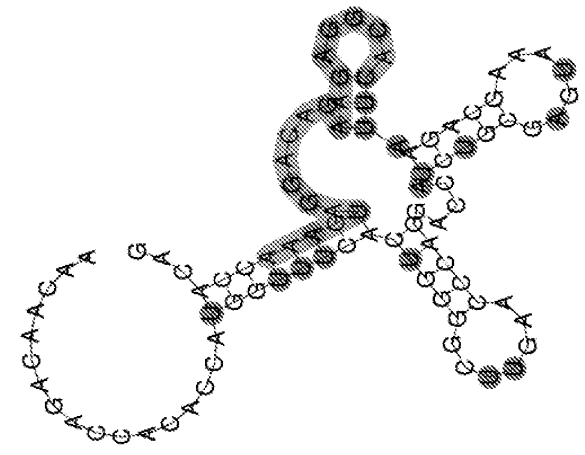

Wild-type miR100
SEQ ID NO: 256
GEiGS-based on miR100 (small nt change)
SEQ ID NO: 257
GEiGS-based on miR100 (large nt change)
SEQ ID NO: 258

FIG. 12C



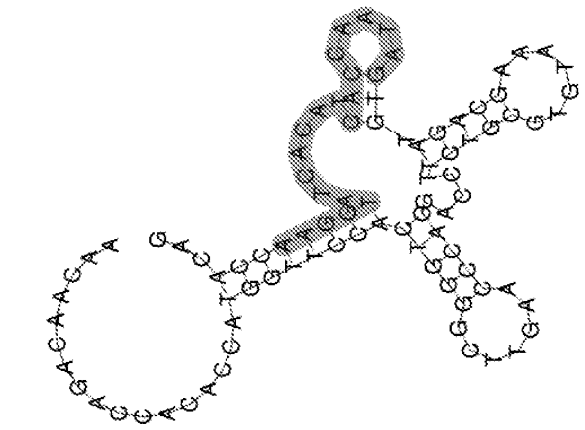
GEiGS-based on tRNA (large nt change)

SEQ ID NO: 261



GEiGS-based on tRNA (small nt change)

SEQ ID NO: 260



Wild-type tRNA

SEQ ID NO: 259

FIG. 12D

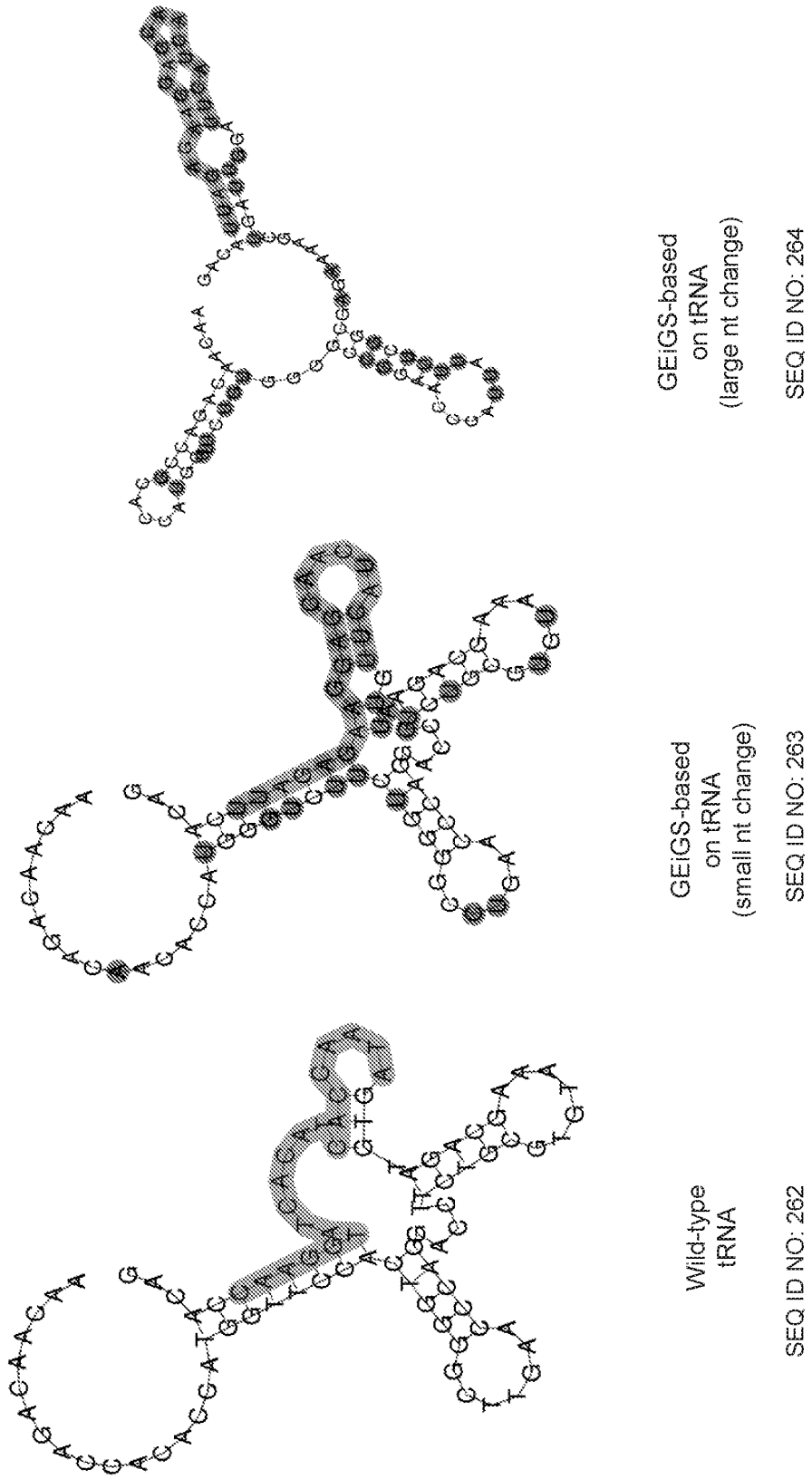

Wild-type tRNA
SEQ ID NO: 262
GEiGS-based on tRNA (small nt change)
SEQ ID NO: 263
GEiGS-based on tRNA (large nt change)
SEQ ID NO: 264

FIG. 12E

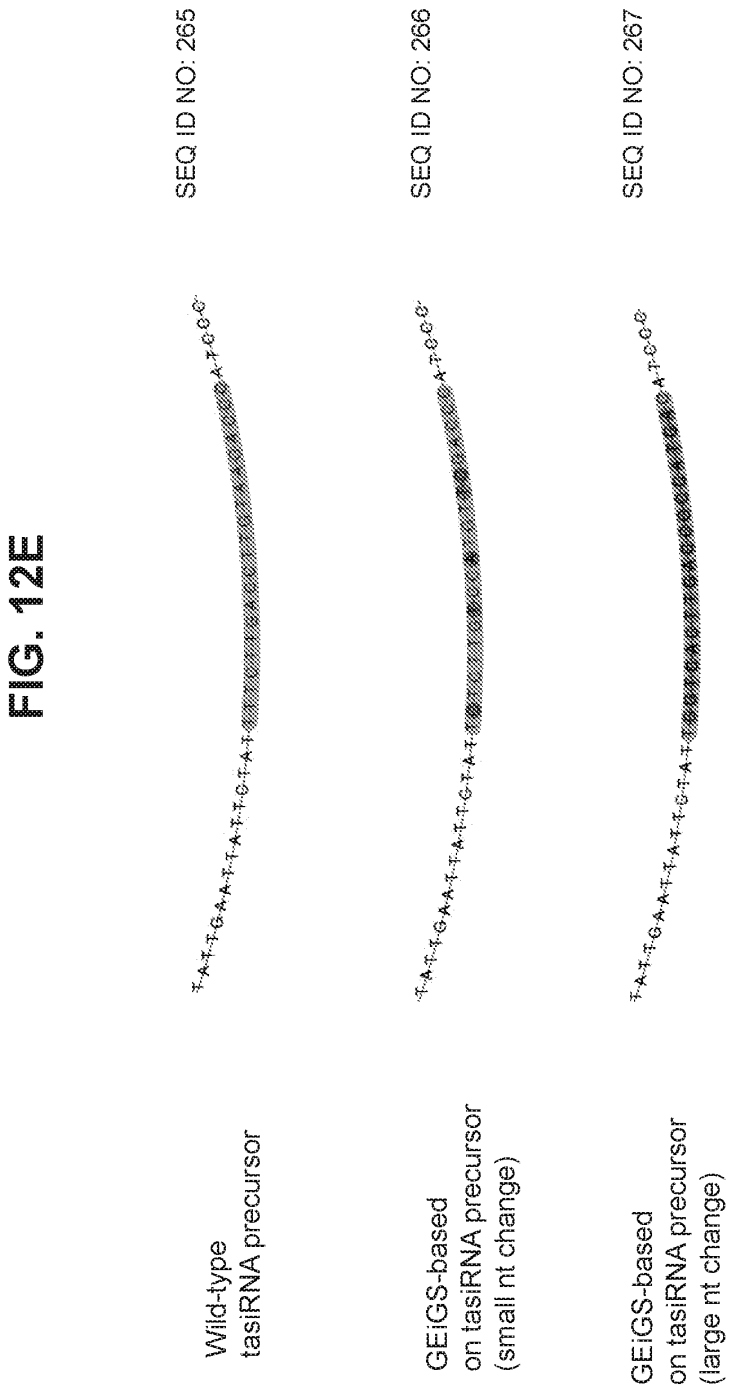

SEQ ID NO: 265
SEQ ID NO: 266
SEQ ID NO: 267
Wild-type tasiRNA precursor
GEiGS-based on tasiRNA precursor (small nt change)
GEiGS-based on tasiRNA precursor (large nt change)

FIG. 13

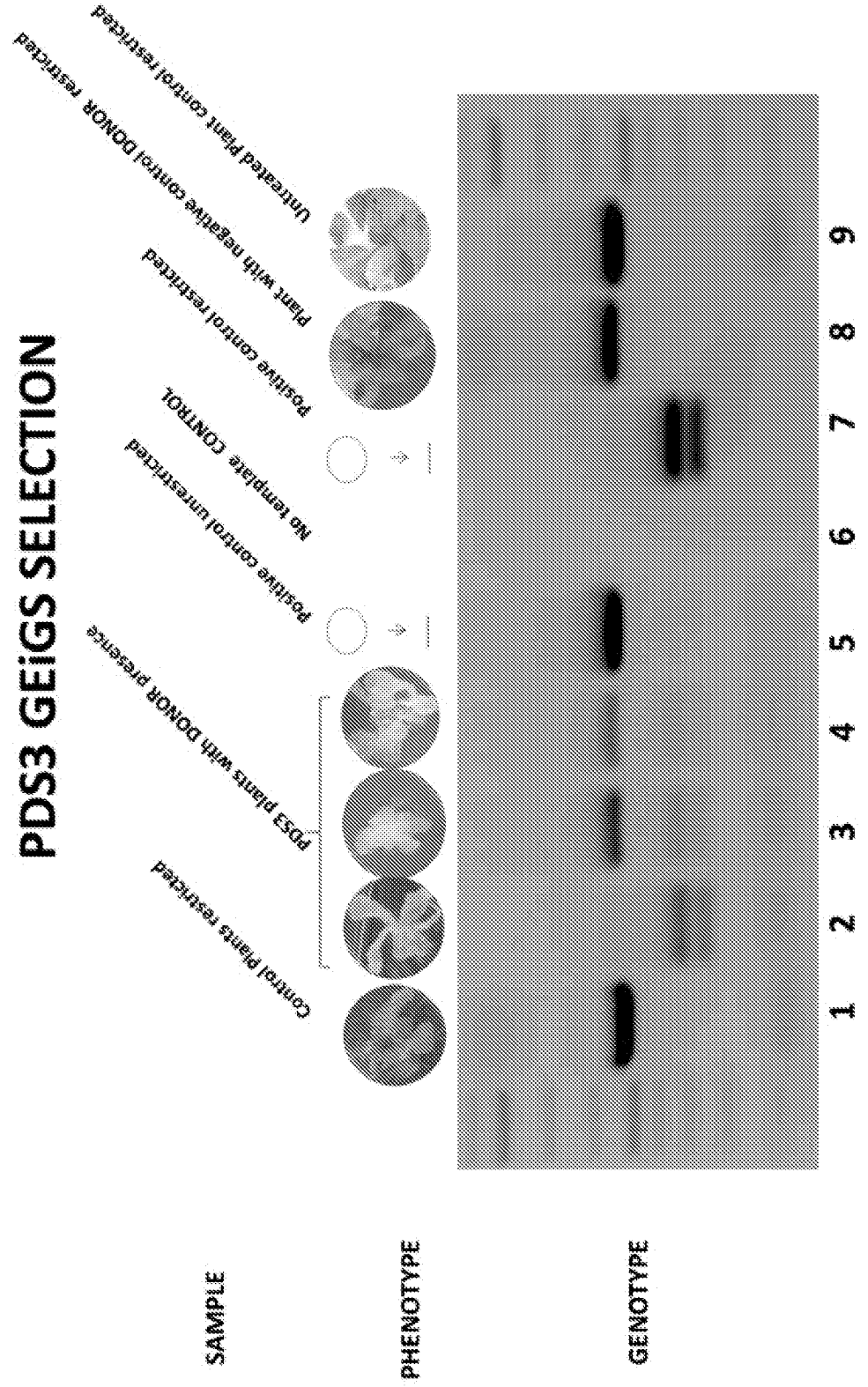

PDS3 GEiGS SELECTION
SAMPLE
PHENOTYPE
GENOTYPE
Control plants restricted
PDS3 plants with DONOR presence
Positive control unrestricted
No template CONTROL
Positive control restricted
Plant with negative control DONOR restricted
Untreated plant control restricted
1 2 3 4 5 6 7 8 9

FIG. 14

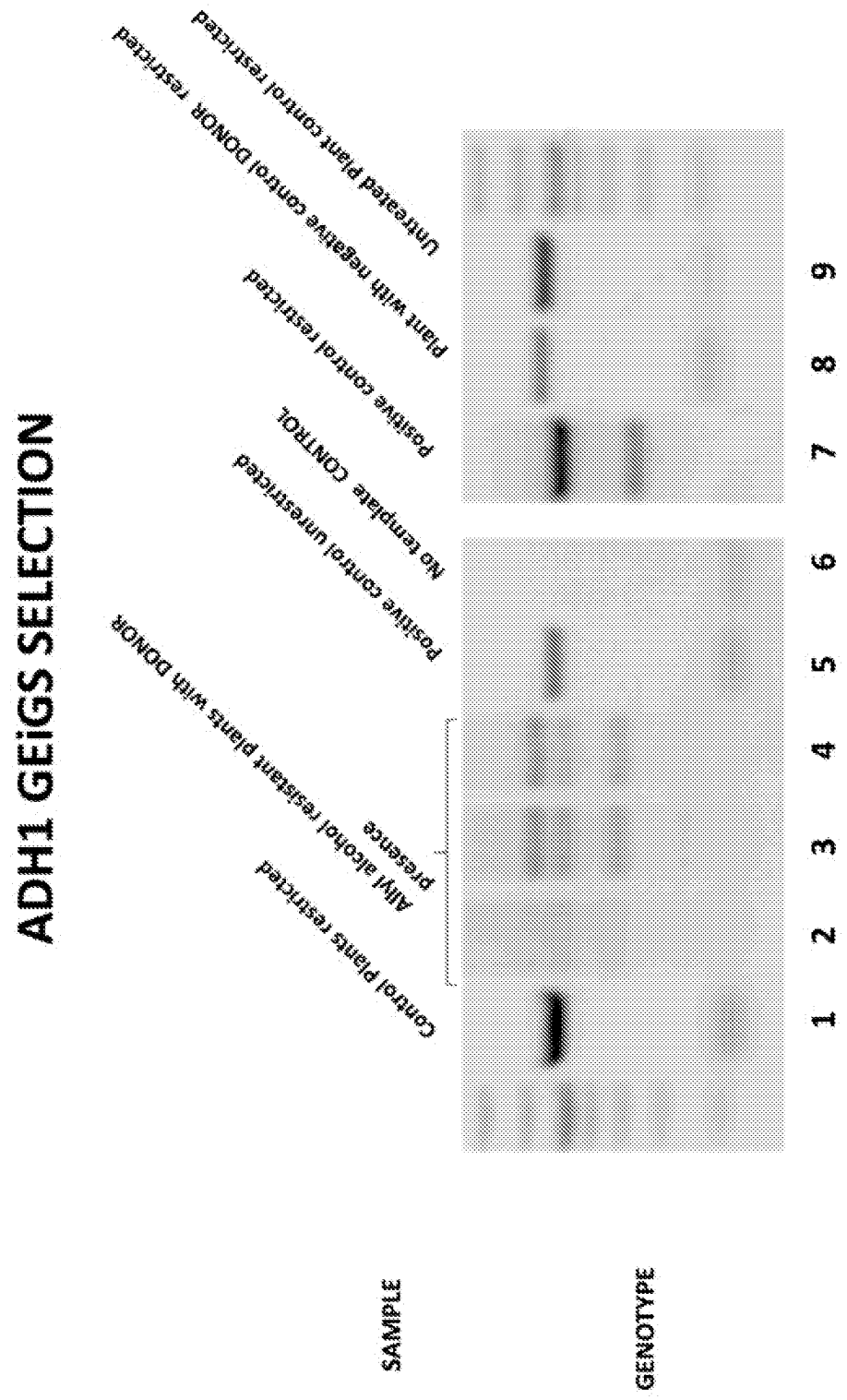

ADH1 GEiGS SELECTION
SAMPLE
GENOTYPE
1 2 3 4 5 6 7 8 9
Control plants resistant
Allyl alcohol resistant plants with DONOR
presence
Positive control unrestricted
No template CONTROL
Positive control restricted
Plant with negative control DONOR restricted
Untreated plant control restricted

FIG. 15

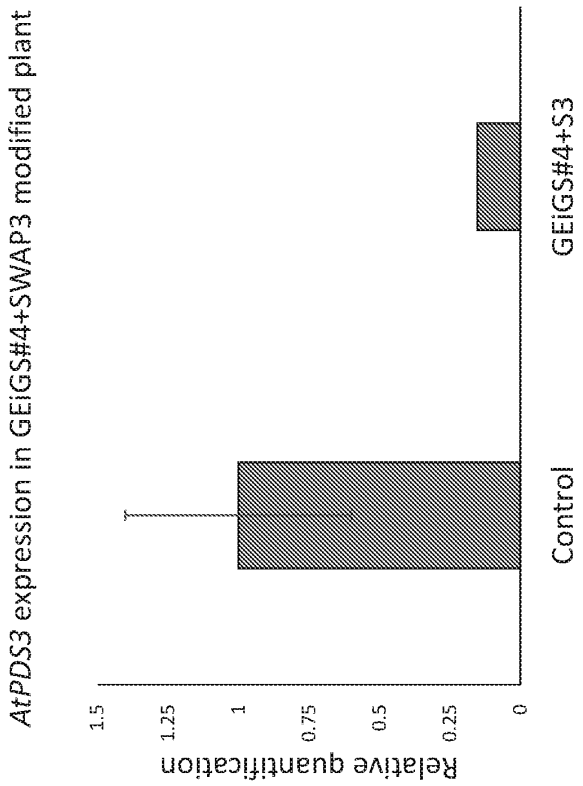
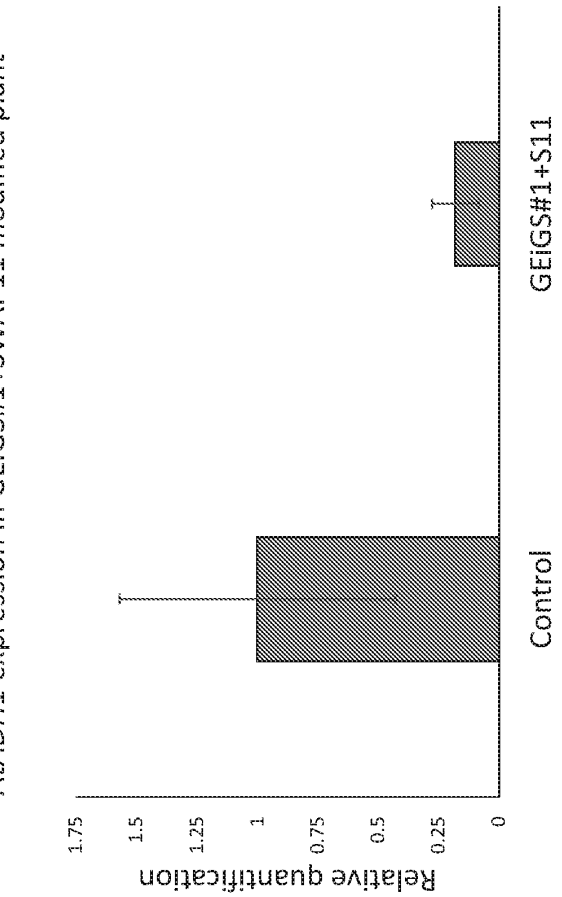

AtPDS3 expression in GEiGS#4+SWAP3 modified plant
Relative quantification
1.5
1.25
1
0.75
0.5
0.25
0
Control
GEiGS#4+S3

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