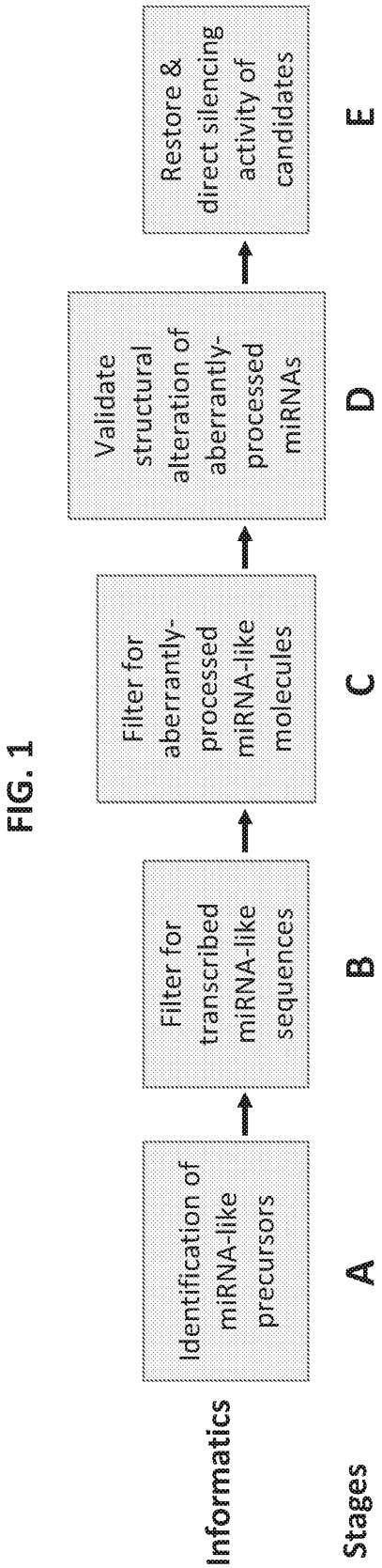

Informatics
Stages
A
Identification of miRNA-like precursors
B
Filter for transcribed miRNA-like sequences
C
Filter for aberrantly-processed miRNA-like molecules
D
Validate structural alteration of aberrantly-processed miRNAs
E
Restore & direct silencing activity of candidates

FIG. 1

FIG. 2

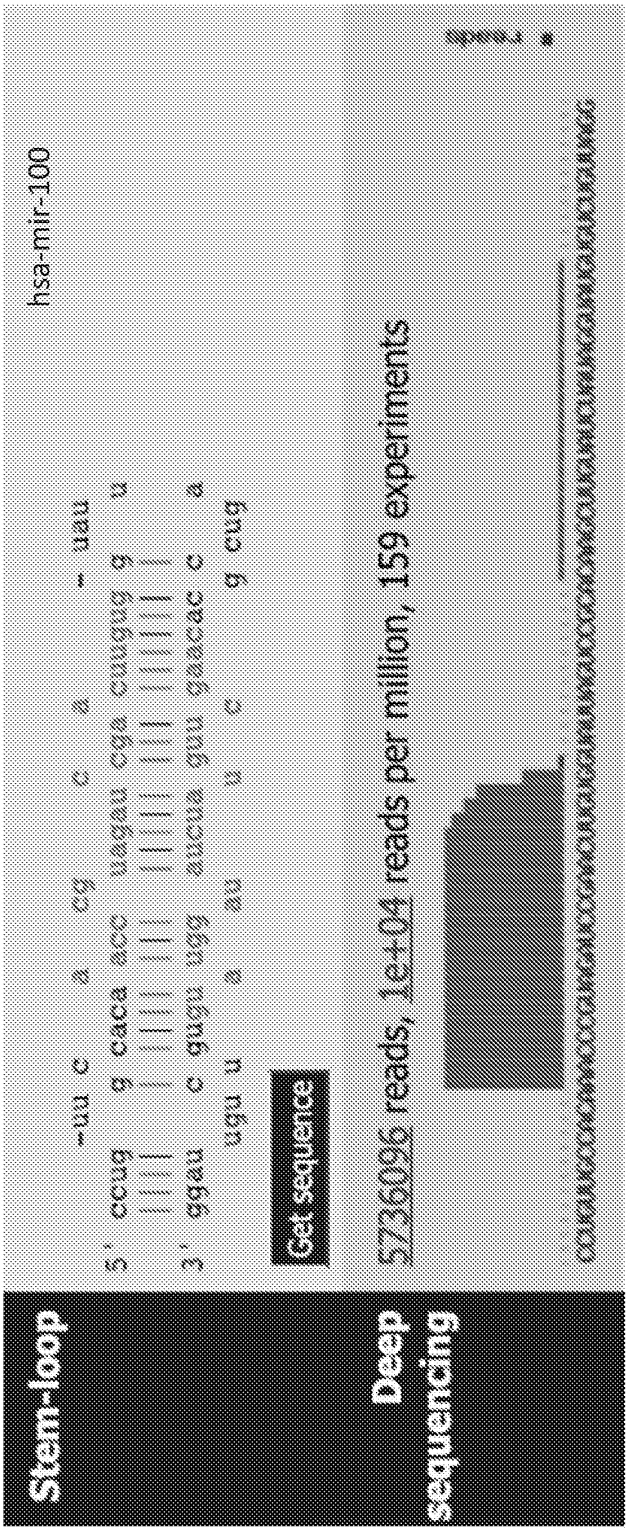

Stem-loop
hsa-mir-100
Cat sequence
Deep sequencing
5736096 reads, 1e+04 reads per million, 159 experiments

FIG. 3

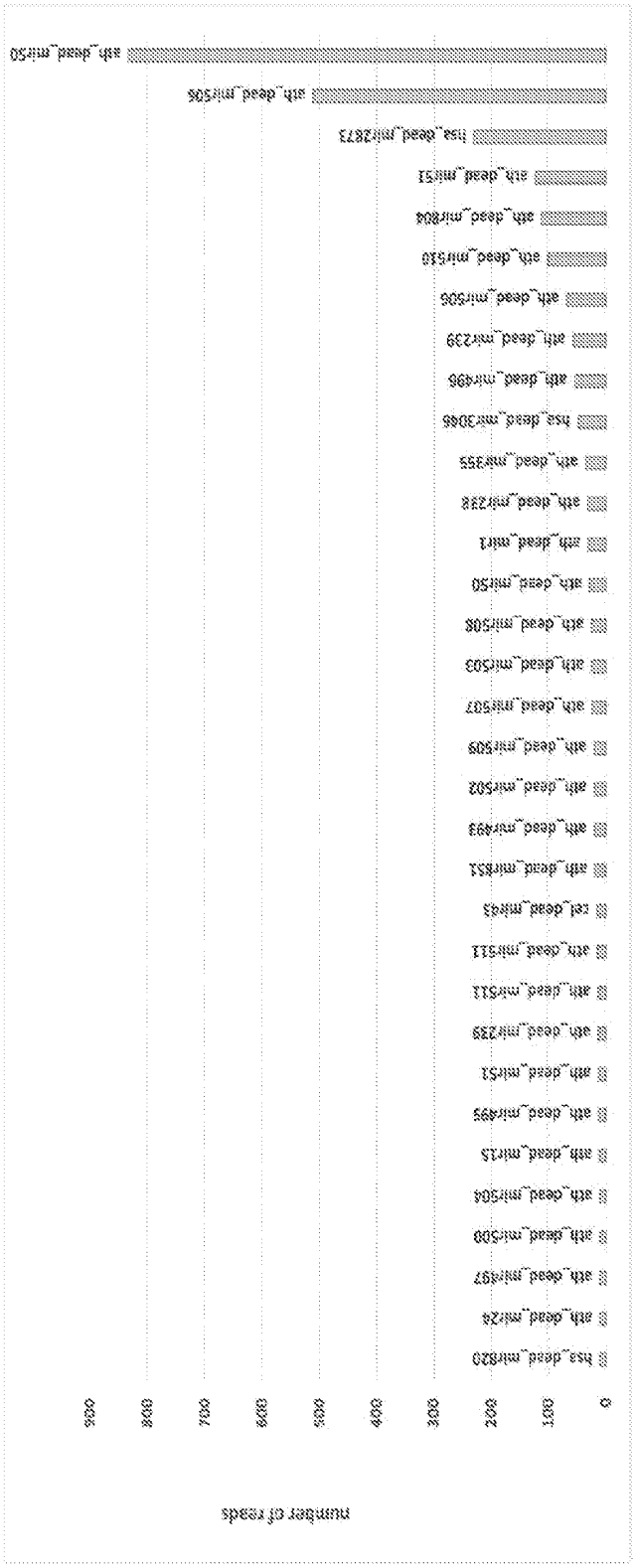

number of reads
0
100
200
300
400
500
600
700
800
hsa_dead_mir820
ath_dead_mir24
ath_dead_mir497
ath_dead_mir500
ath_dead_mir504
ath_dead_mir15
ath_dead_mir495
ath_dead_mir51
ath_dead_mir239
ath_dead_mir511
ath_dead_mir311
ath_dead_mir43
ref_dead_mir43
ath_dead_mir851
ath_dead_mir493
ath_dead_mir502
ath_dead_mir509
ath_dead_mir507
ath_dead_mir503
ath_dead_mir508
ath_dead_mir50
ath_dead_mir1
ath_dead_mir238
ath_dead_mir355
hsa_dead_mir3088
ath_dead_mir498
ath_dead_mir239
ath_dead_mir506
ath_dead_mir510
ath_dead_mir804
ath_dead_mir51
hsa_dead_mir2873
ath_dead_mir506
ath_dead_mir50

FIG. 4

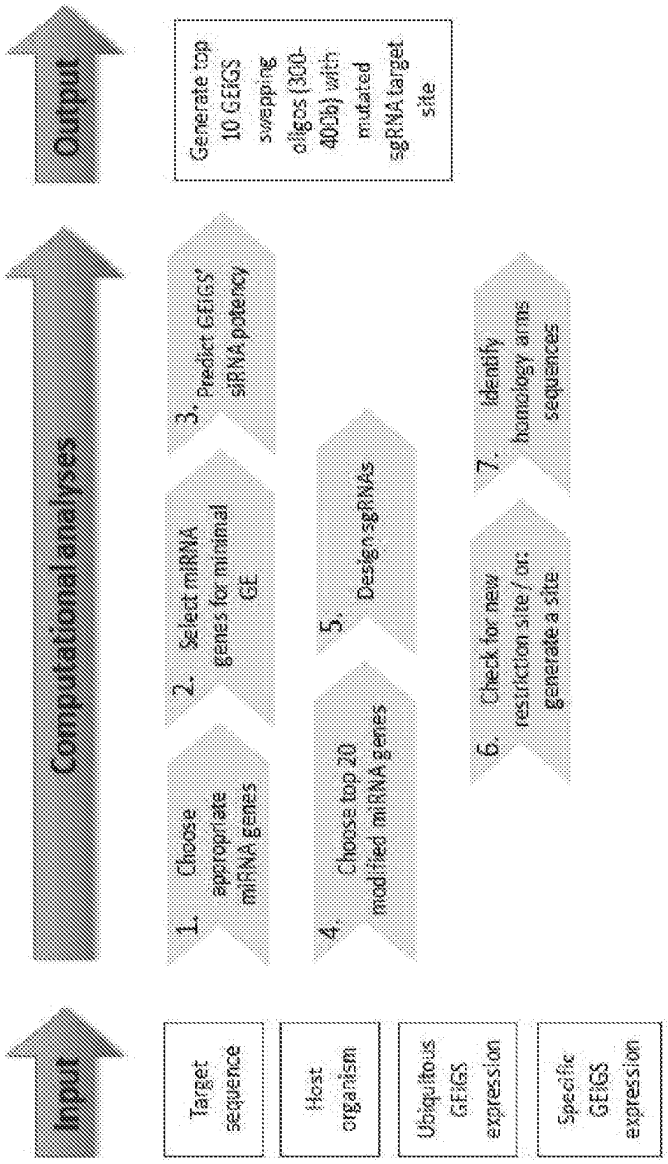
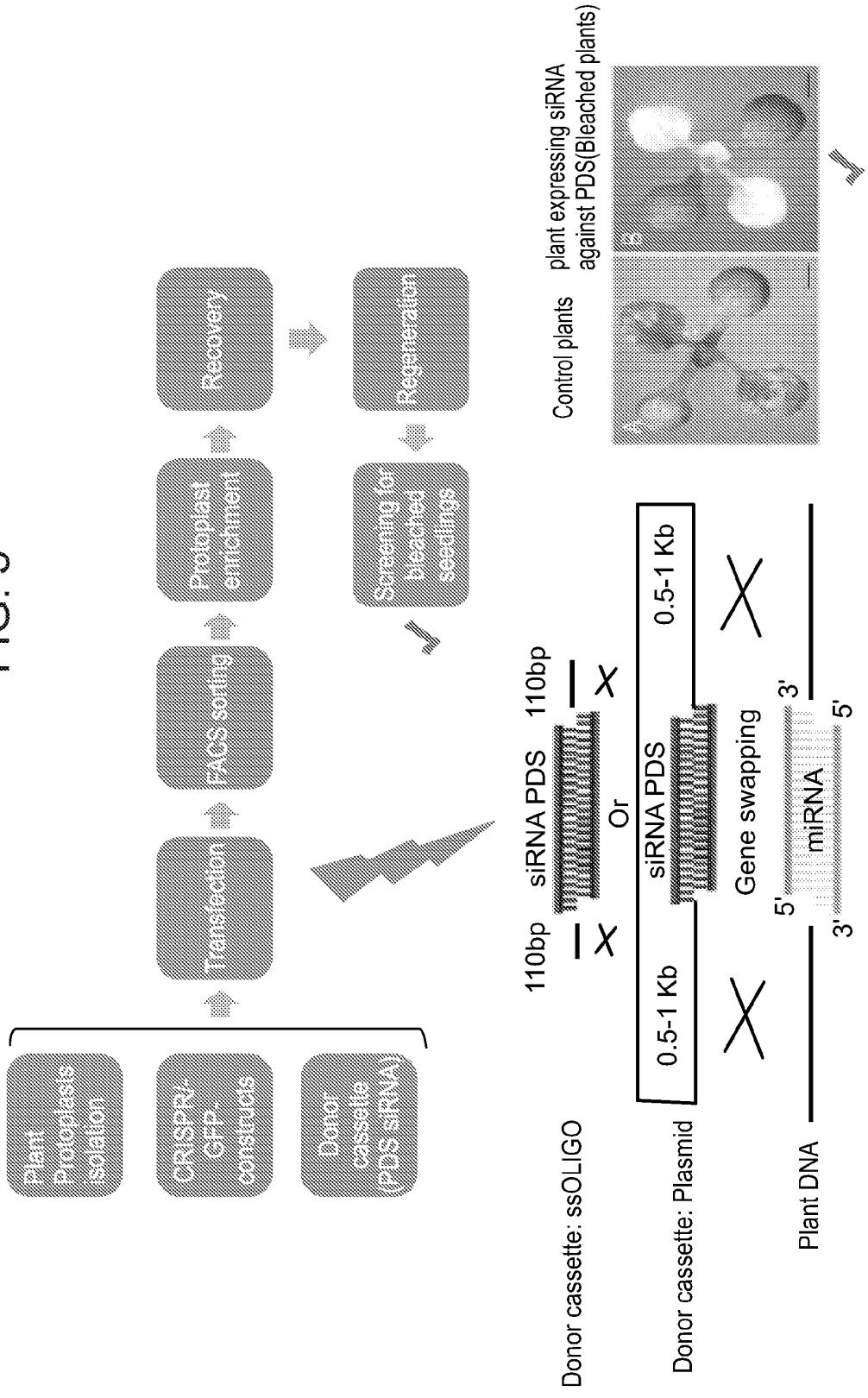

Input
Target sequence
Host organism
Ubiquitous GEIGS expression
Specific GEIGS expression
Computational analyses
1. Choose appropriate miRNA genes
2. Select miRNA genes for minimal GE
3. Predict GEIGS' siRNA potency
4. Choose top 20 modified miRNA genes
5. Design sgRNAs
6. Check for new restriction site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

FIG. 5


Plant Protoplasts isolation
CRISPR/Cas9 constructs
Donor cassette (PDS siRNA)
Transfection
FACS sorting
Protoplast entrainment
Recovery
Regeneration
Screening for bleached seedlings
Control plants
plant expressing siRNA against PDS(Bleached plants)
110bp
siRNA PDS
110bp
Or
Donor cassette: ssOLIGO
0.5-1 Kb
siRNA PDS
0.5-1 Kb
Donor cassette: Plasmid
Gene swapping
5'
3'
miRNA
3'
5'
Plant DNA

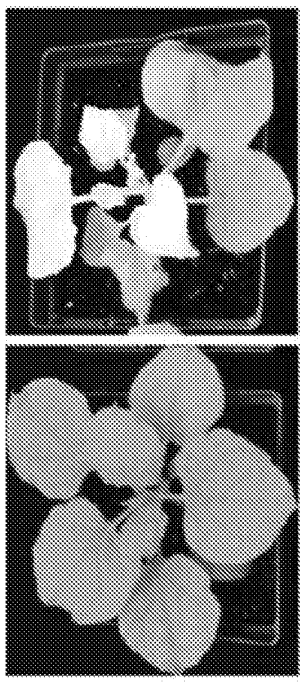


FIG. 6A

FIG. 6B

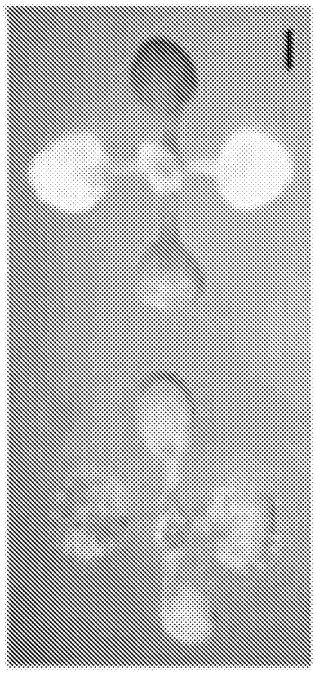


FIG. 6C

FIG. 7

Transcript
Secondary structure
Mature sRNA
From miRBase
wt
Homologous sequence to the wt
Dead
wt- based coordinates
Introduction of sequence modifications that result in changes in secondary structure to resemble the wt secondary structure and give rise to a sRNA from the same coordinates as the one from the wt precursor
Reactivated
wt- based coordinates
Introduction of sequence modifications on the reactivated version that maintain the secondary structure but result in the maturation of sRNA that targets PDS3
Anti-PDS
Anti-PDS
Redirected
Anti-PDS

FIG. 8A

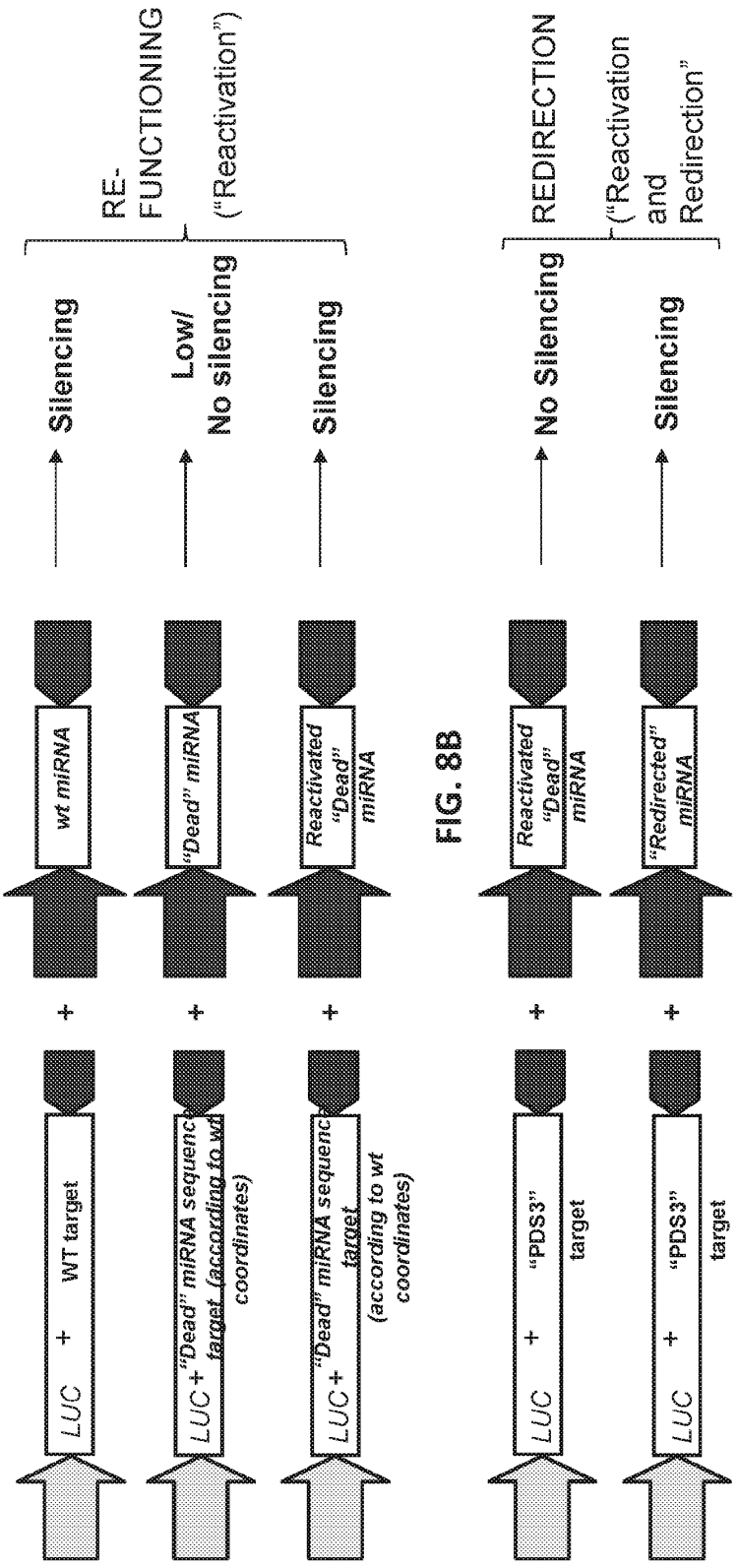

Luc-sensor vector
GEiGS-oligo vector
Expected observation
LUC + WT target
wt miRNA
Silencing
LUC + "Dead" miRNA sequenc target (according to wt coordinates)
"Dead" miRNA
Low/ No silencing
LUC + "Dead" miRNA sequenc target (according to wt coordinates)
Reactivated "Dead" miRNA
Silencing
RE-FUNCTIONING ("Reactivation")

FIG. 8B

LUC + "PDS3" target
Reactivated "Dead" miRNA
No Silencing
LUC + "PDS3" target
"Redirected" miRNA
Silencing
REDIRECTION ("Reactivation and Redirection")

FIG. 9A

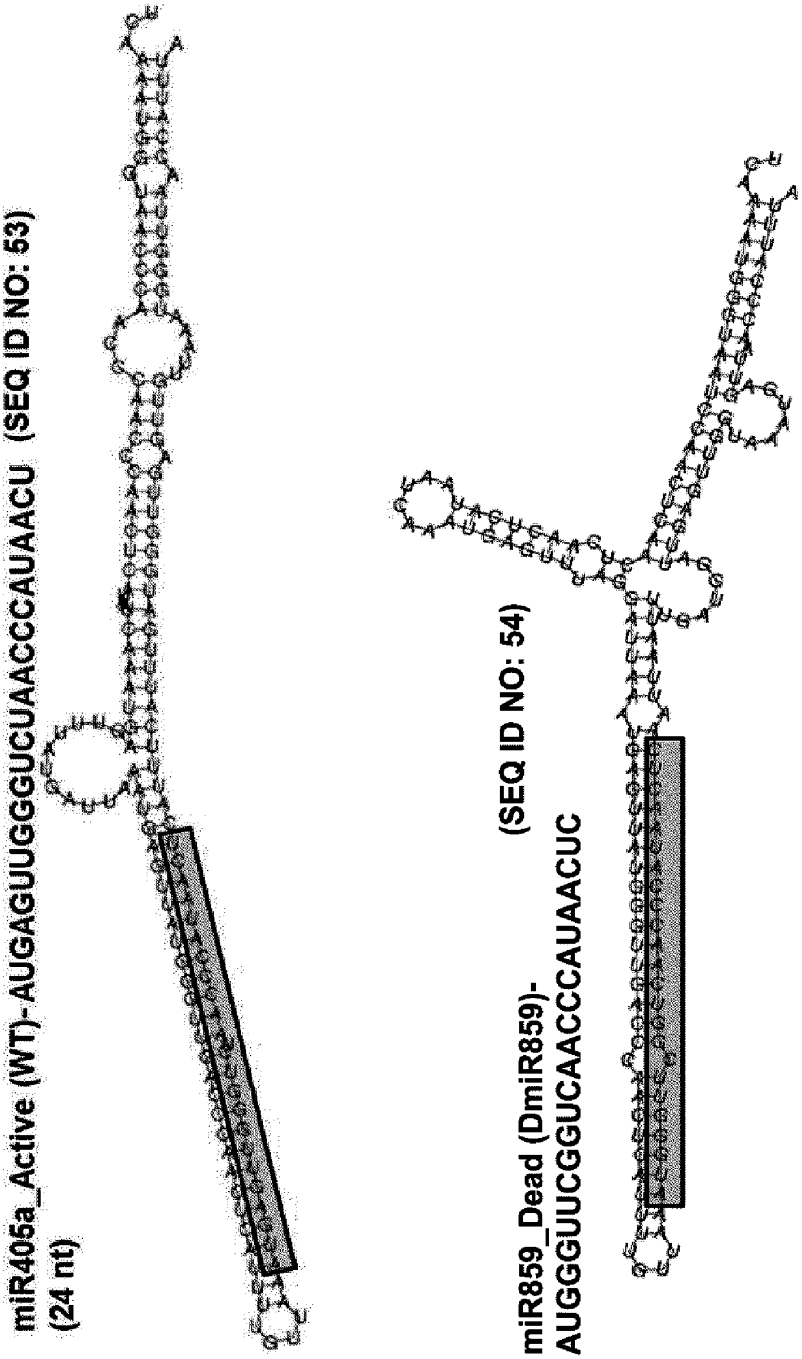

miR405a_Active (WT)-AUGAGUUGGGUCUAACCCAUAACU (SEQ ID NO: 53)
(24 nt)
miR859_Dead (DmiR859)-
AUGGGUUCGGUCAACCCAUAACUC
(SEQ ID NO: 54)

FIG. 9A
continuation

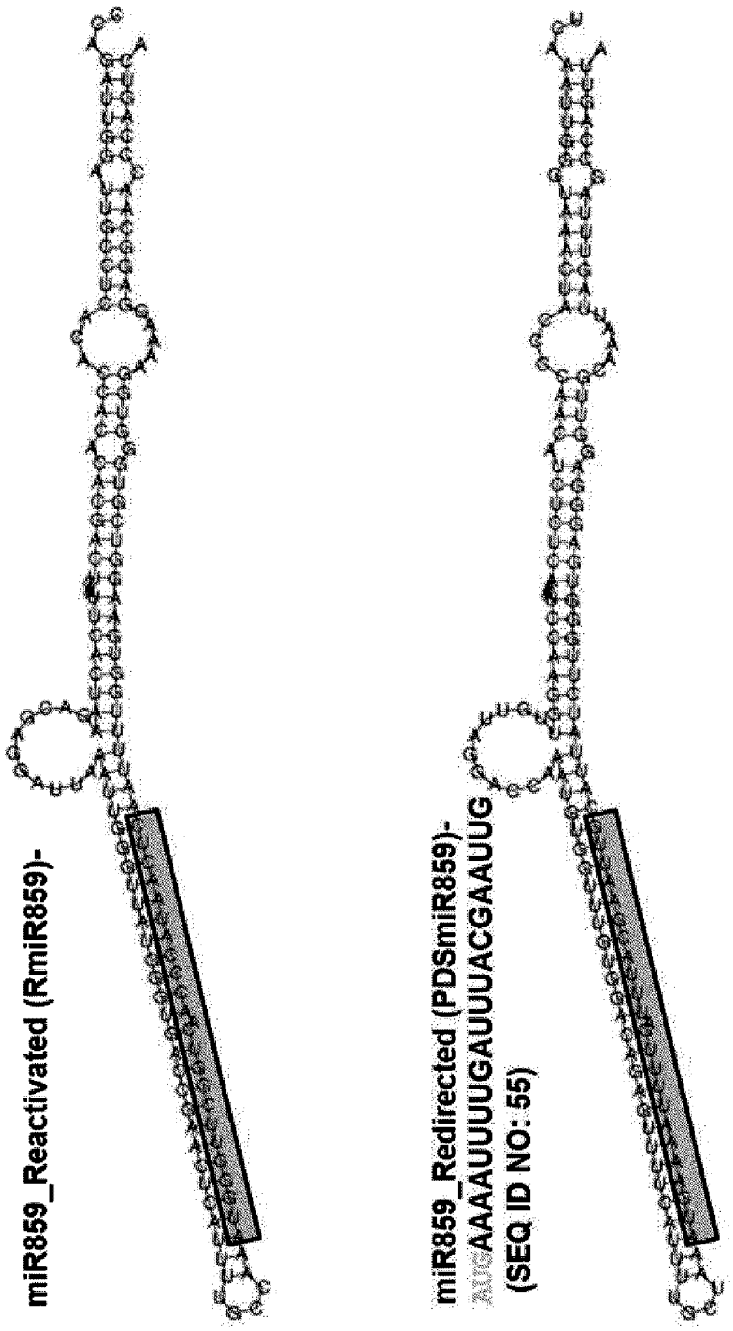


FIG. 9B

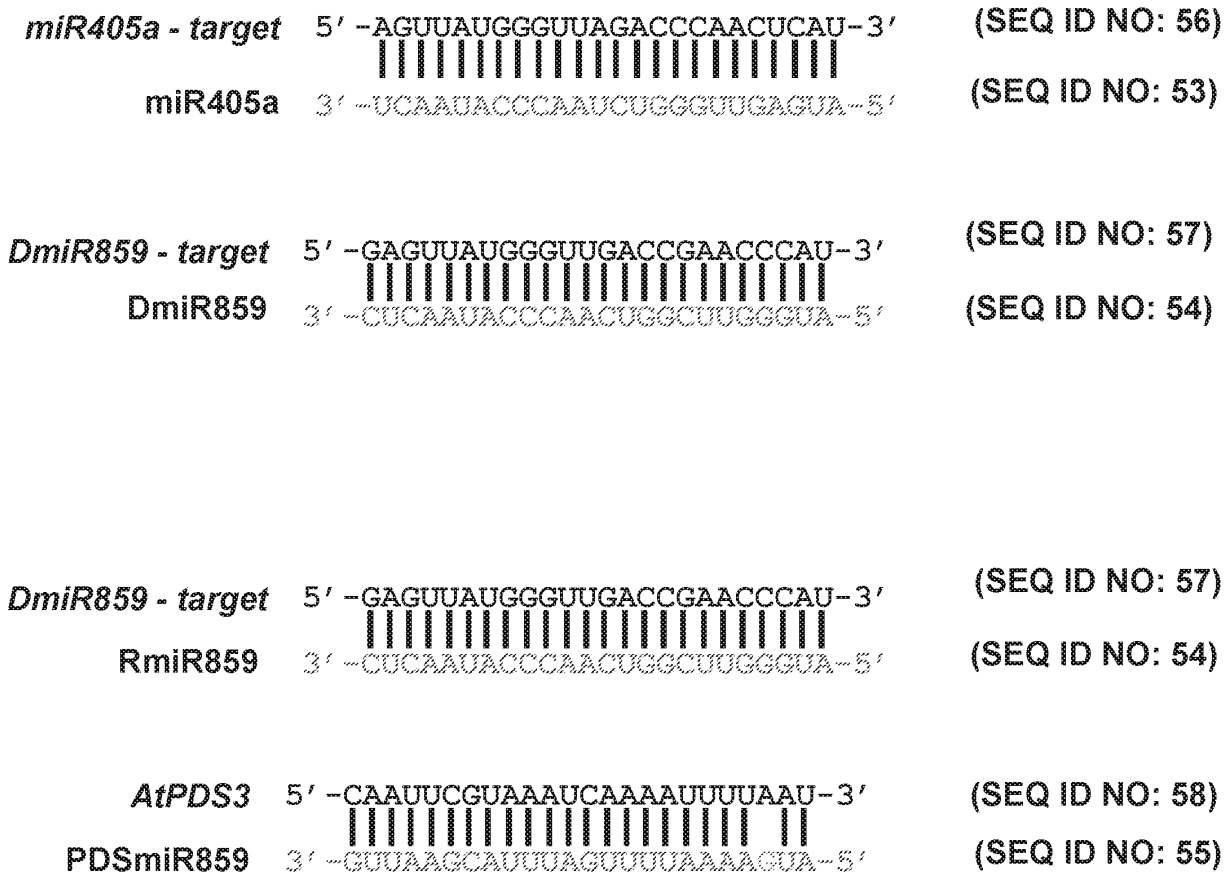

miR405a - target
5′ -AGUUAUGGGUUAGACCCAACUCAU-3′
(SEQ ID NO: 56)
miR405a
3′ -UCAAUACCCAAUCUGGGUUGAGUA-5′
(SEQ ID NO: 53)
DmiR859 - target
5′ -GAGUUAUGGGUUGACCGAACCCAU-3′
(SEQ ID NO: 57)
DmiR859
3′ -CUCAAUACCCAACUGGCUUGGGUA-5′
(SEQ ID NO: 54)
DmiR859 - target
5′ -GAGUUAUGGGUUGACCGAACCCAU-3′
(SEQ ID NO: 57)
RmiR859
3′ -CUCAAUACCCAACUGGCUUGGGUA-5′
(SEQ ID NO: 54)
AtPDS3
5′ -CAAUUCGUAAAUCAAAAUUUUAAU-3′
(SEQ ID NO: 58)
PDSmiR859
3′ -GUUAAGCAUUUAGUUUUAAAAGUA-5′
(SEQ ID NO: 55)

FIG. 9C

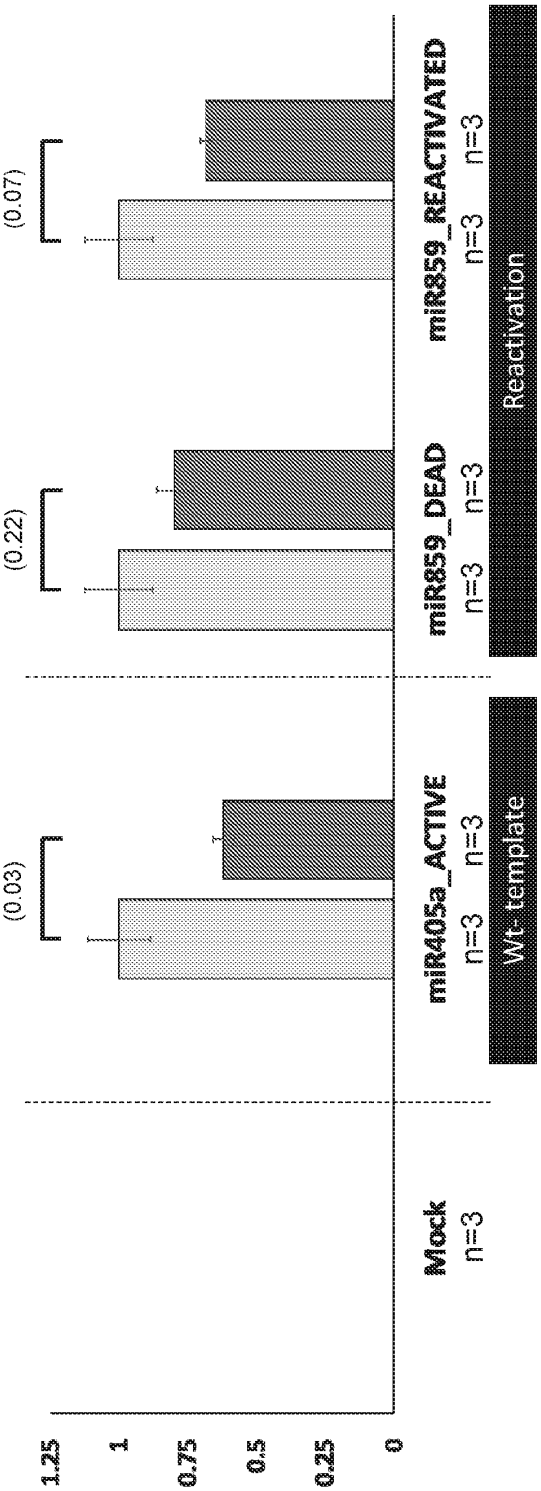

Ratio fLUC/eGFP Col-0 protoplasts - 3 Days Post Treatment
(0.03)
(0.22)
(0.07)
1.25
1
0.75
0.5
0.25
0
Mock
n=3
miR405a_ACTIVE
n=3
n=3
miR859_DEAD
n=3
n=3
miR859_REACTIVATED
n=3
n=3
Wt- template
Reactivation

FIG. 9D

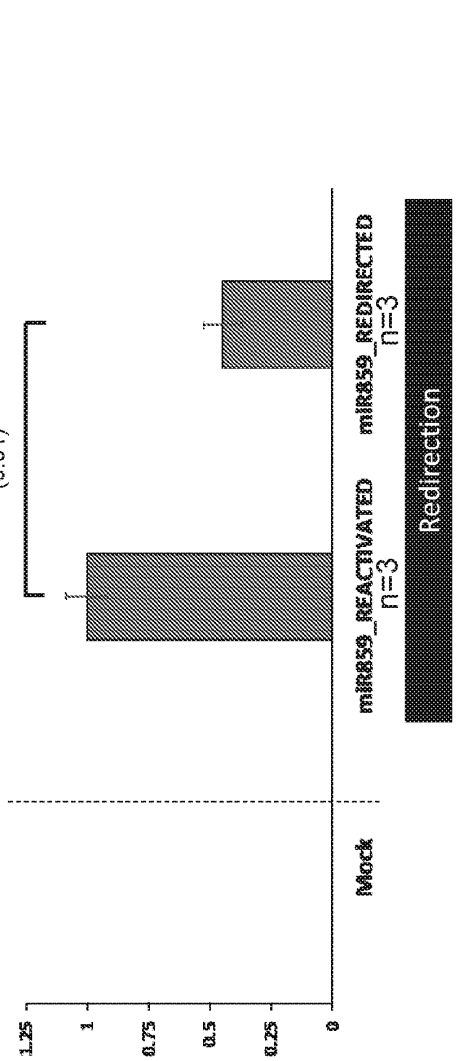

Ratio fLUC/eGFP Col-0 protoplasts - 3 Days Post Treatment
(0.01)
1.25
1
0.75
0.5
0.25
0
Mock
miR859_REACTIVATED
n=3
miR859_REDIRECTED
n=3
Redirection

FIG. 9E

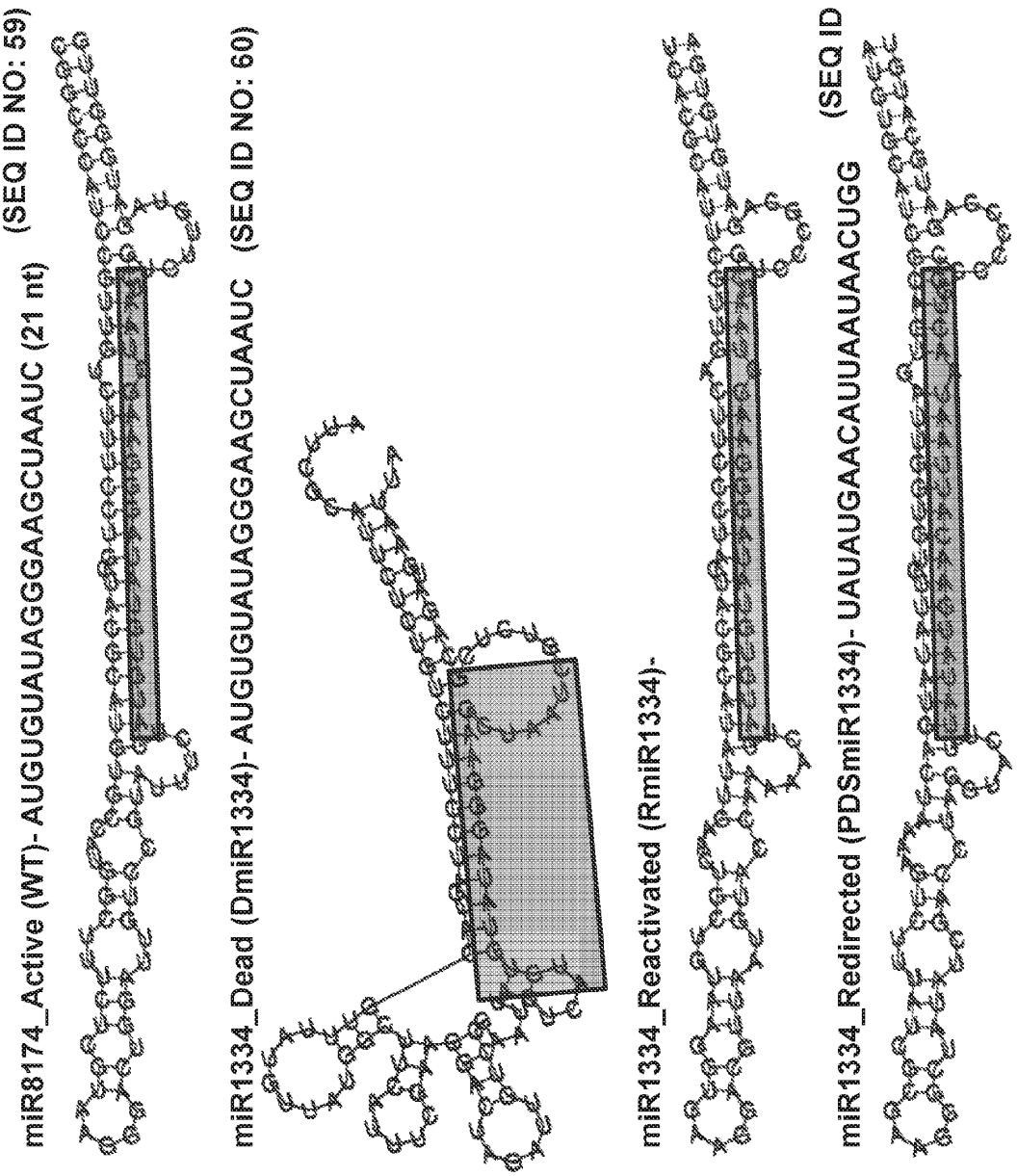

miR8174_Active (WT)- AUGUGUAUAGGGAAGCUAAUC (21 nt) (SEQ ID NO: 59)
miR1334_Dead (DmiR1334)- AUGUGUAUAGGGAAGCUAAUC (SEQ ID NO: 60)
miR1334_Reactivated (RmiR1334)-
miR1334_Redirected (PDSmiR1334)- UAUAUGAACAUUAAUAACUGG (SEQ ID NO: 61)

FIG. 9F

miR8174 - target
5′ –GAUUAGCUUCCCUAUACACAU– 3′
(SEQ ID NO: 62)
miR8174
3′ –CUAAUCGAAGGGAUAUGUGUA– 5′
(SEQ ID NO: 59)
DmiR1334 - target
5′ –GAUUAGCUUCCCUAUACACAU– 3′
(SEQ ID NO: 63)
DmiR1334
3′ –CUAAUCGAAGGGAUAUGUGUA– 5′
(SEQ ID NO: 60)
DmiR1334 - target
5′ –GAUUAGCUUCCCUAUACACAU– 3′
(SEQ ID NO: 63)
RmiR1334
3′ –CUAAUCGAAGGGAUAUGUGUA– 5′
(SEQ ID NO: 60)
AtPDS3
5′ –CCAGUUAUUAAUGUUCAUAUA– 3′
(SEQ ID NO: 64)
PDSmiR1334
3′ –GGUCAAUAAUUACAAGUAUAU– 5′
(SEQ ID NO: 61)

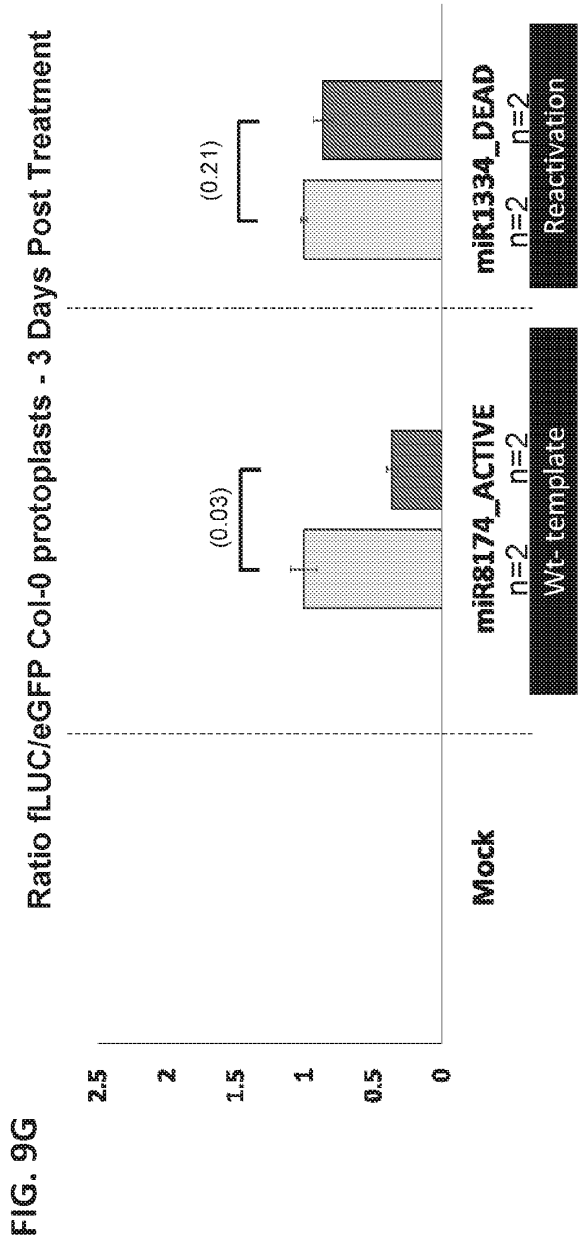

Ratio fLUC/eGFP Col-0 protoplasts - 3 Days Post Treatment
2.5
2
1.5
1
0.5
0
(0.03)
(0.21)
Mock
miR8174_ACTIVE
n=2
n=2
miR1334_DEAD
n=2
n=2
Wt- template
Reactivation

FIG. 9G

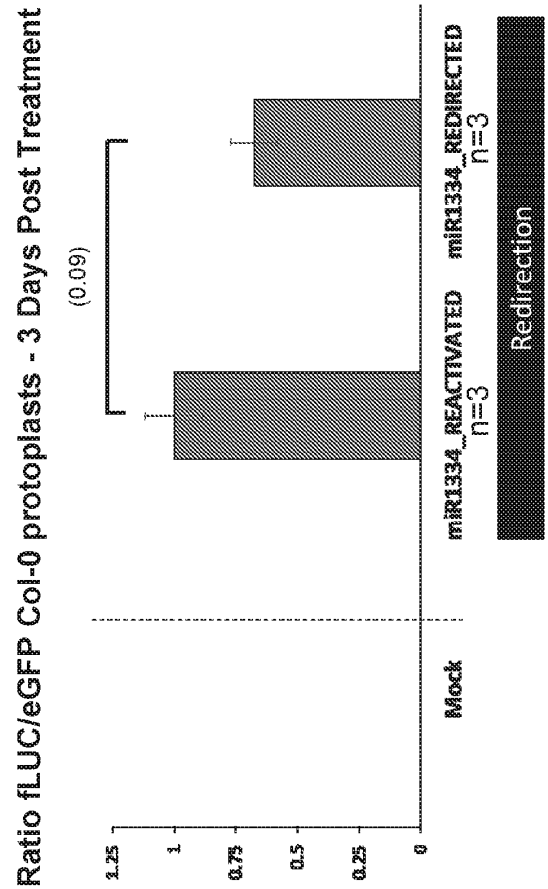

Ratio fLUC/eGFP Col-0 protoplasts - 3 Days Post Treatment
1.25
1
0.75
0.5
0.25
0
(0.09)
Mock
miR1334_REACTIVATED
n=3
miR1334_REDIRECTED
n=3
Redirection

FIG. 9H

FIG. 10A

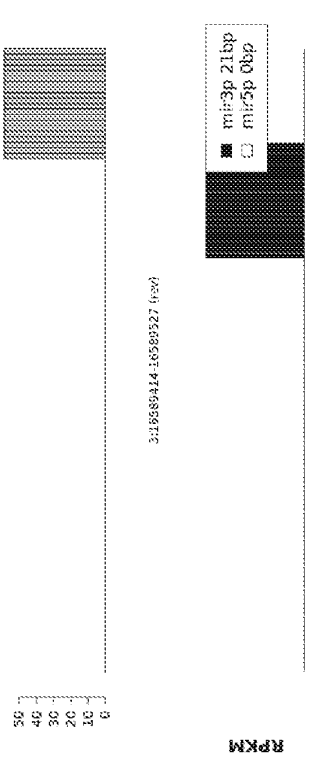


FIG. 10B

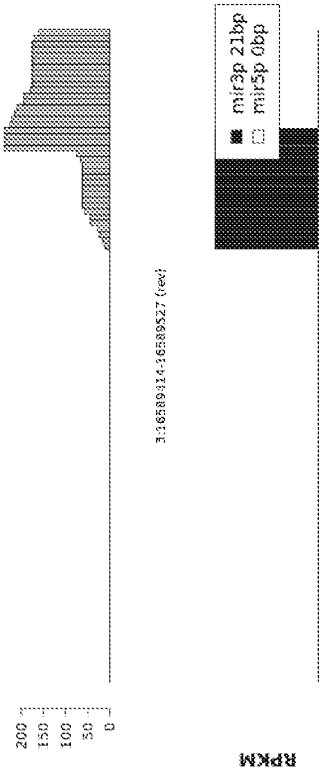


FIG. 10C

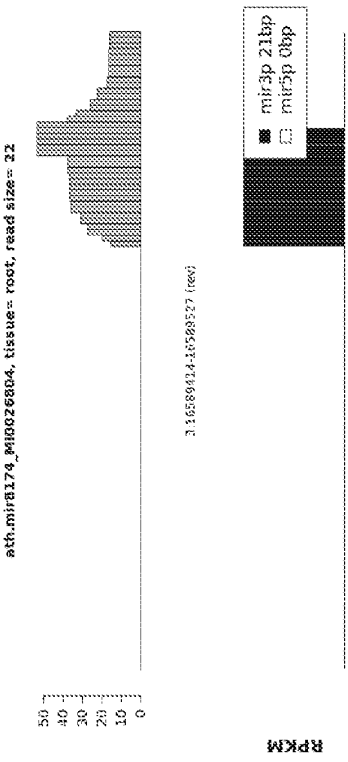


FIG. 10D

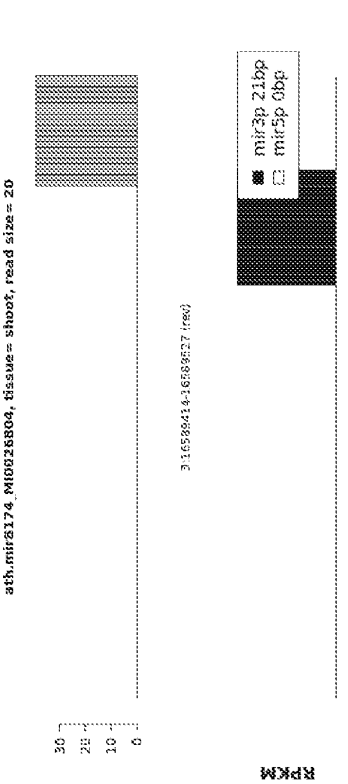


FIG. 10E

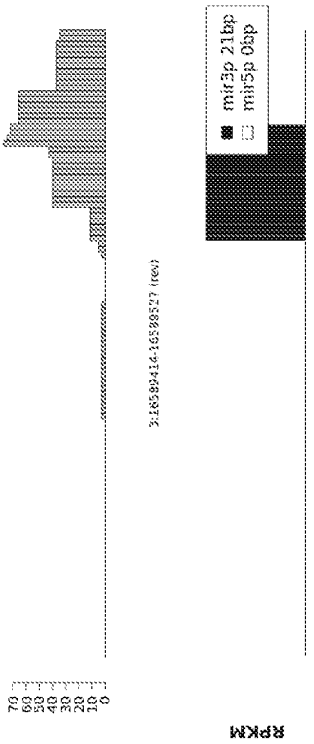


FIG. 10F

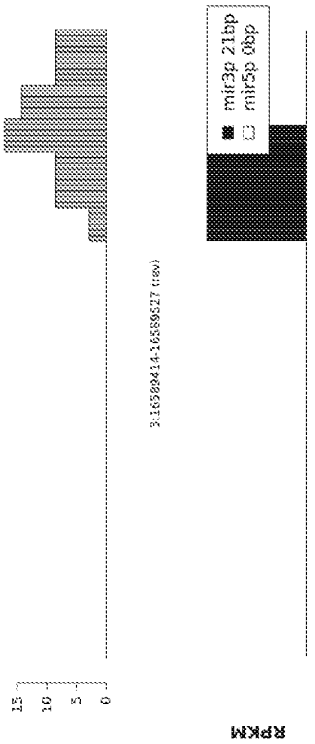


FIG. 10G

ath.mir8174_MI0026804 2D structure

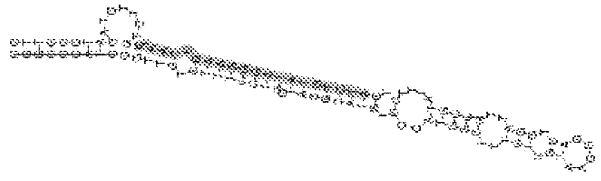


FIG. 10H

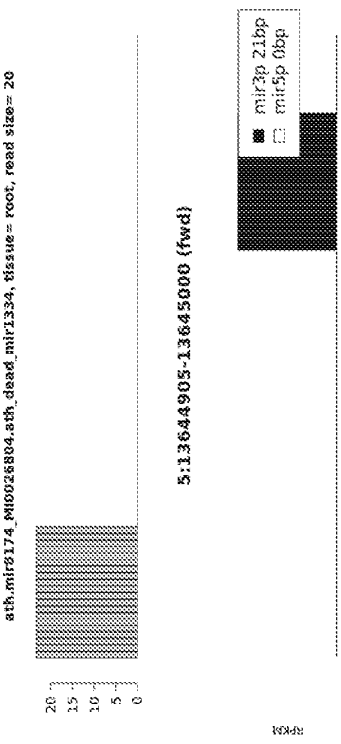


FIG. 10I

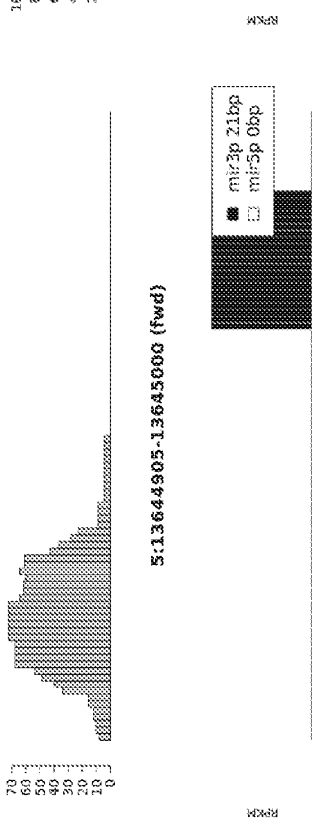


FIG. 10J

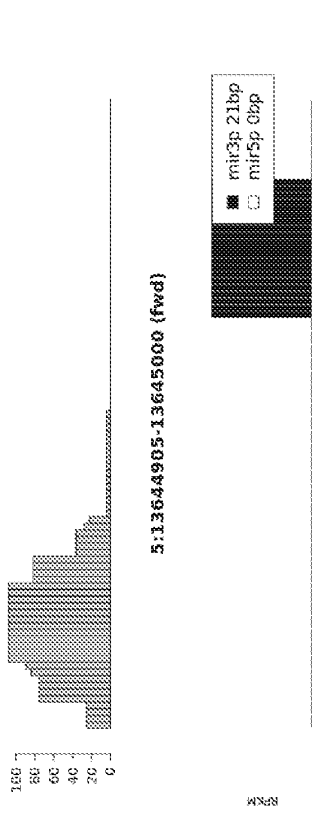


FIG. 10K

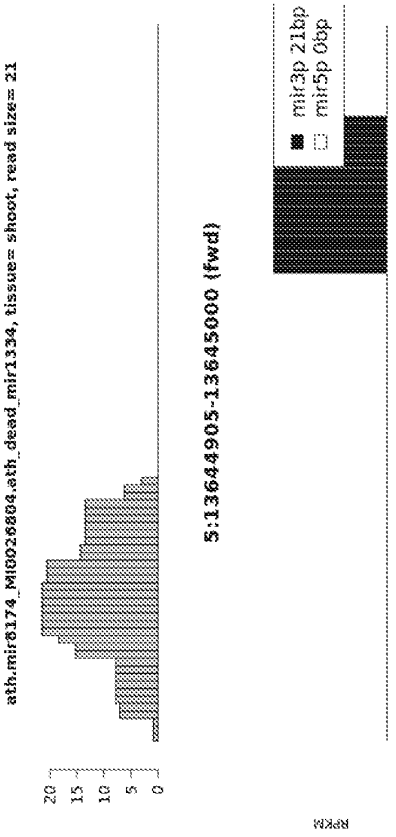


FIG. 10L

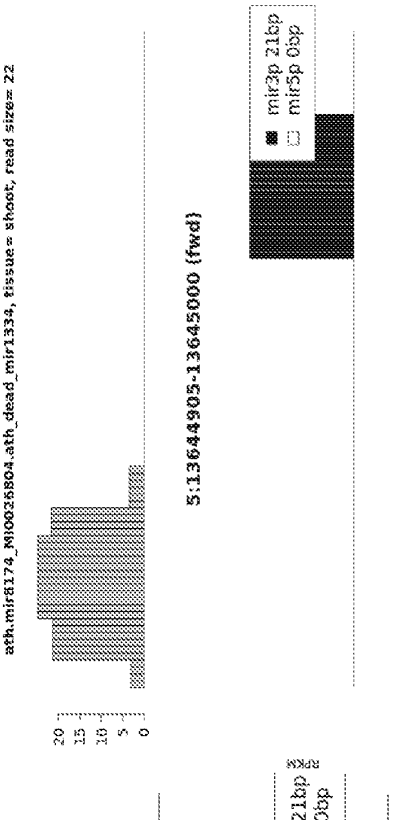


FIG. 10N

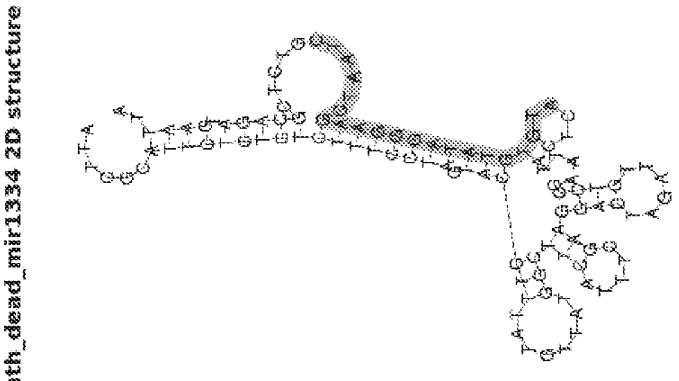


FIG. 10M

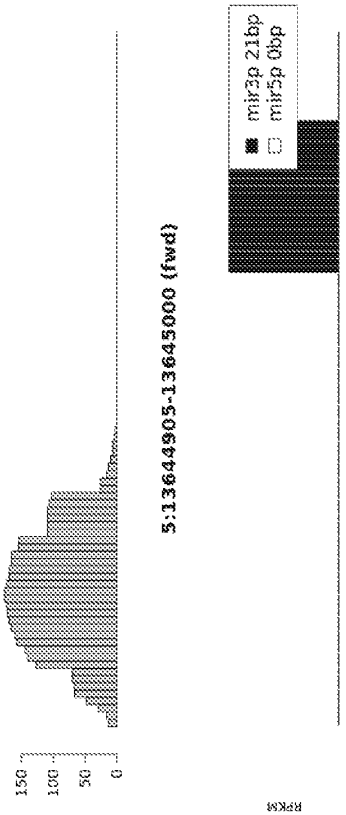


FIG. 11A

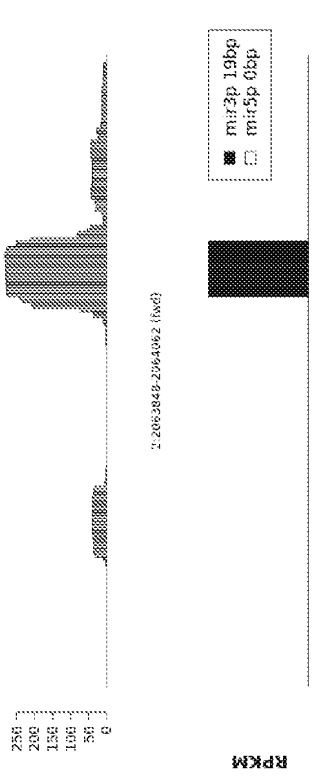


FIG. 11B

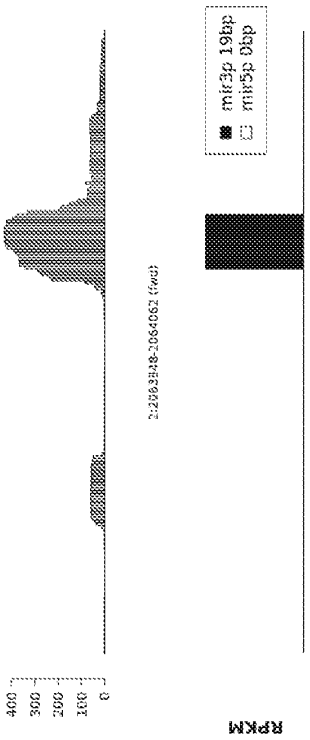


FIG. 11C

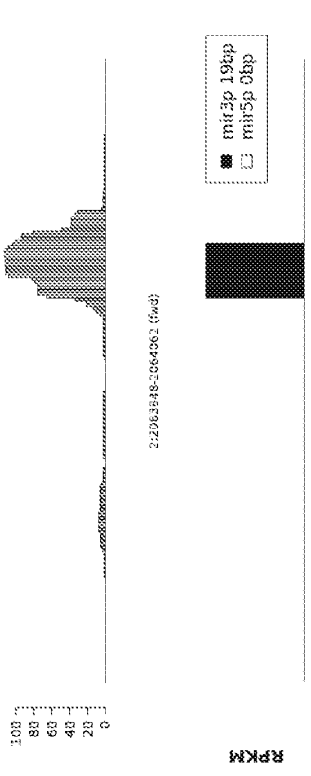


FIG. 11D

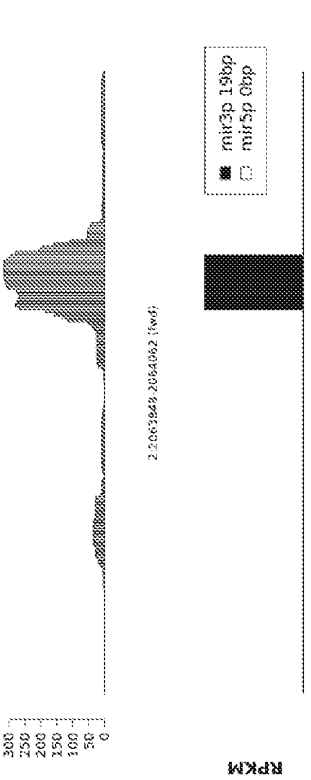


FIG. 11E



FIG. 11F

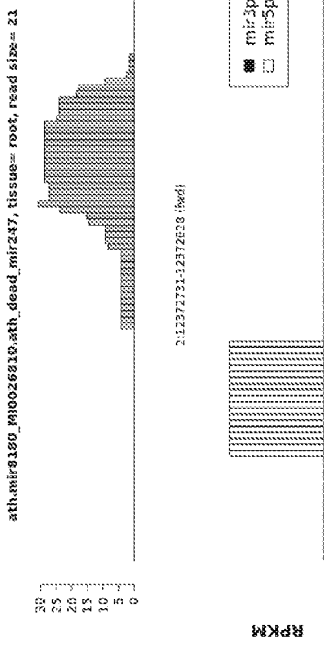


FIG. 11G

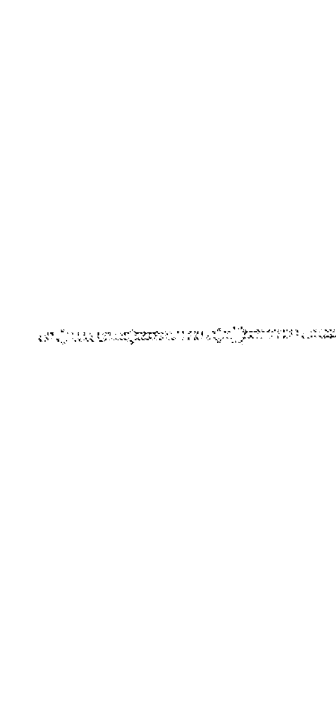


FIG. 11H

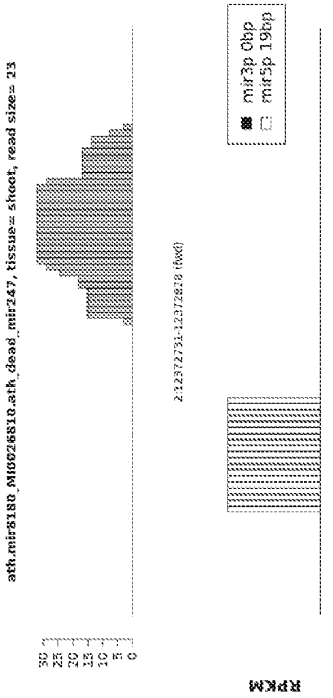


FIG. 11I

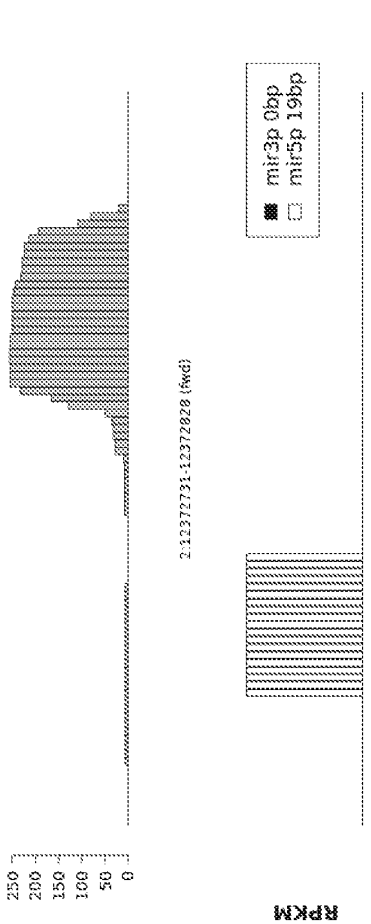


FIG. 11J

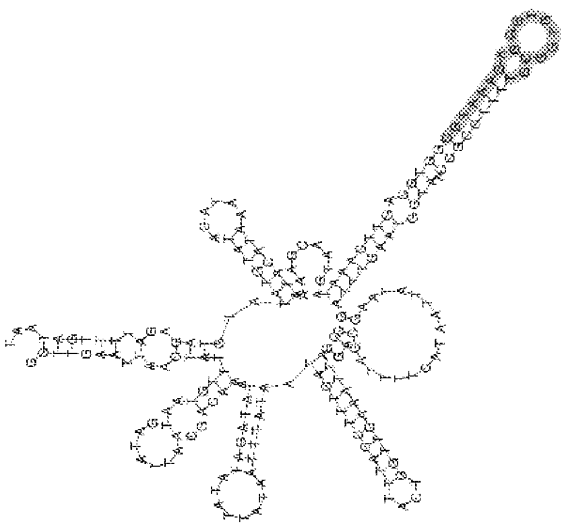


FIG. 12A

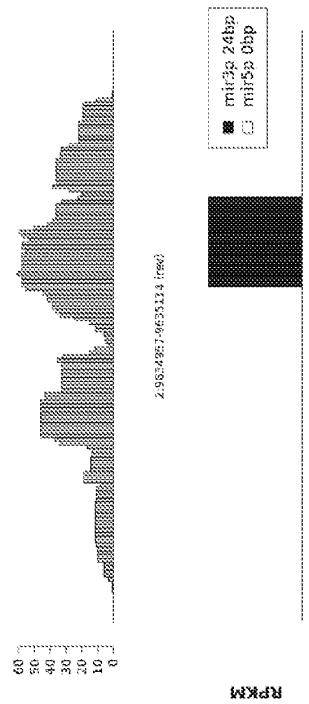


FIG. 12B

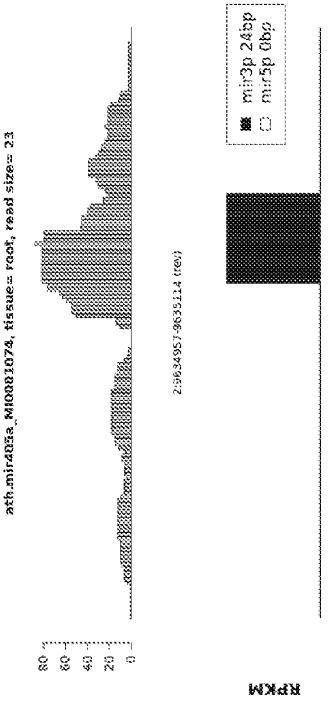


FIG. 12C

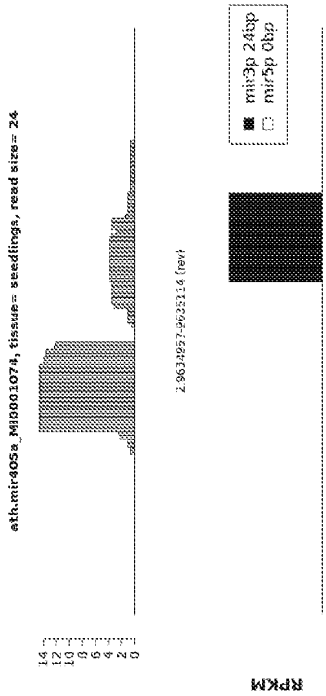


FIG. 12D

ath.mir405a_MI0001074 2D structure

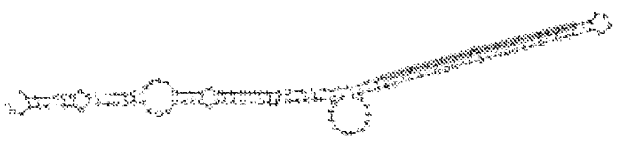


FIG. 12E

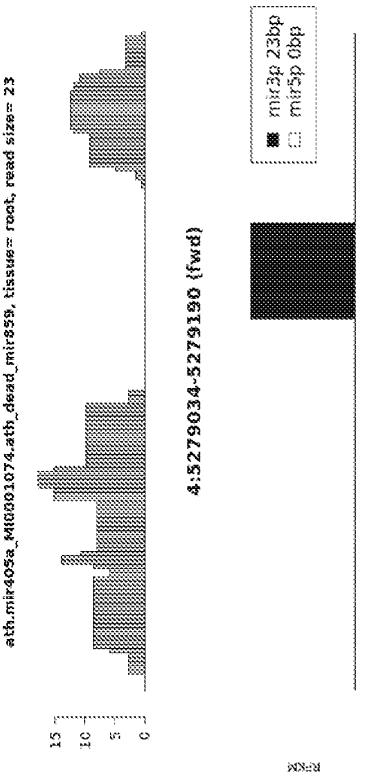


FIG. 12F

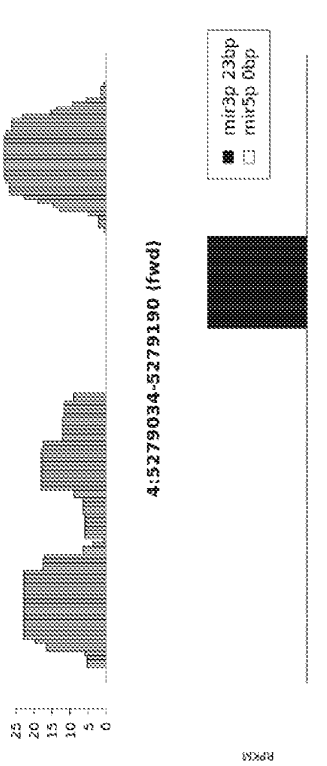


FIG. 12G

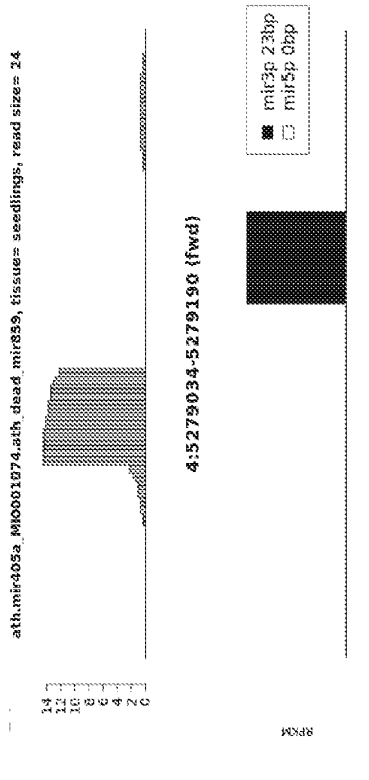


FIG. 12H

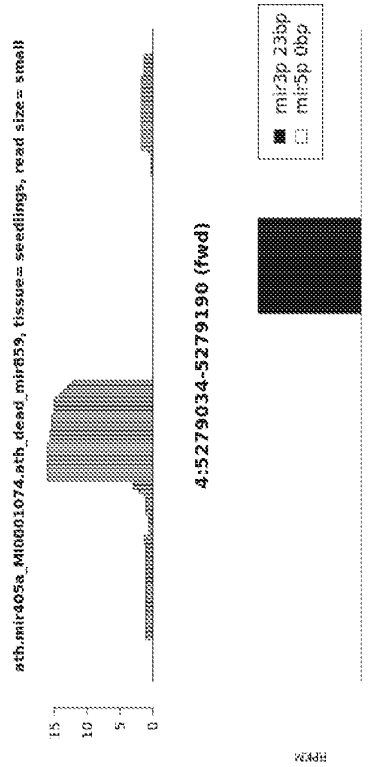


FIG. 12I

ath_dead_mir859 2D structure

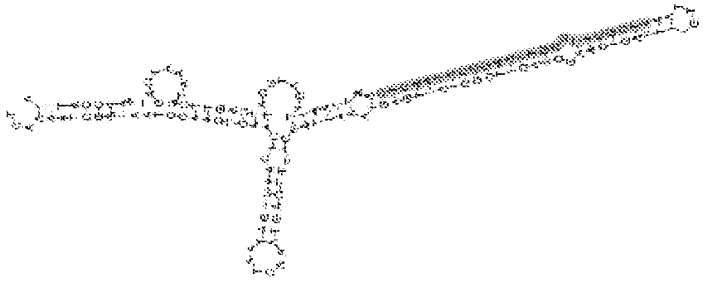


FIG. 13A

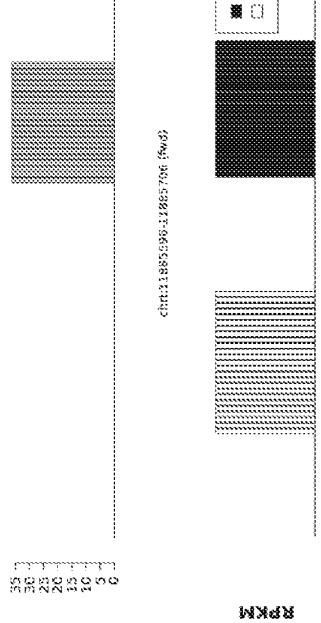


FIG. 13B

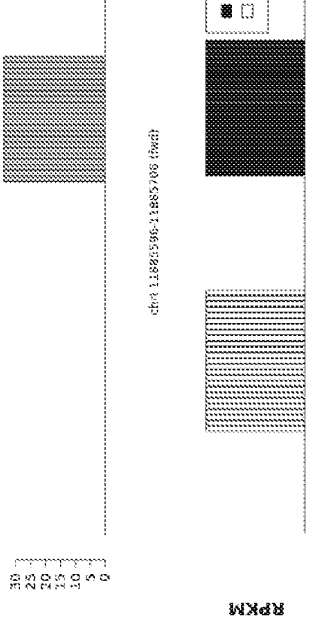


FIG. 13C

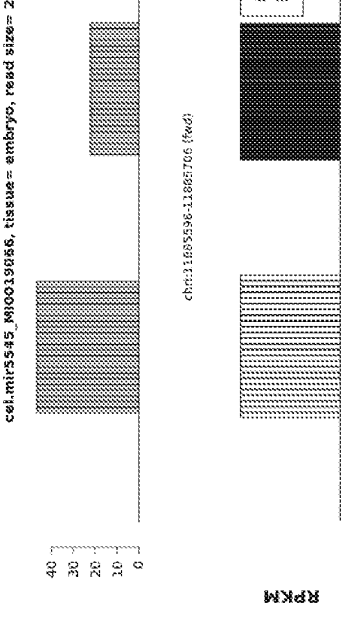


FIG. 13D

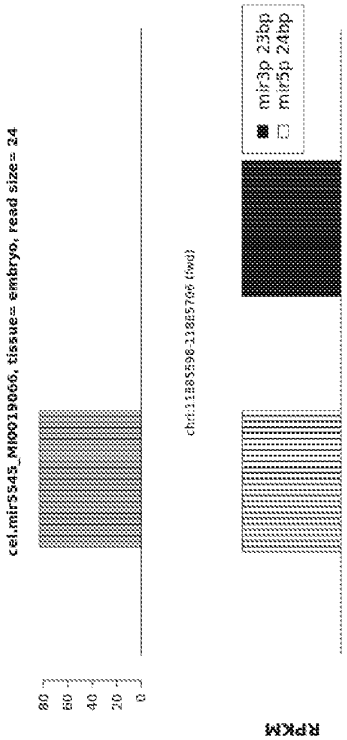


FIG. 13E

cel.mir5545_MI0019066 2D structure

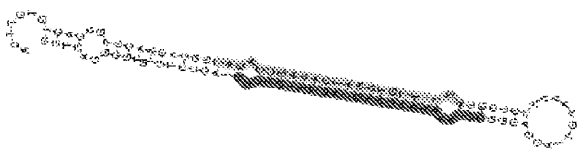


FIG. 13F

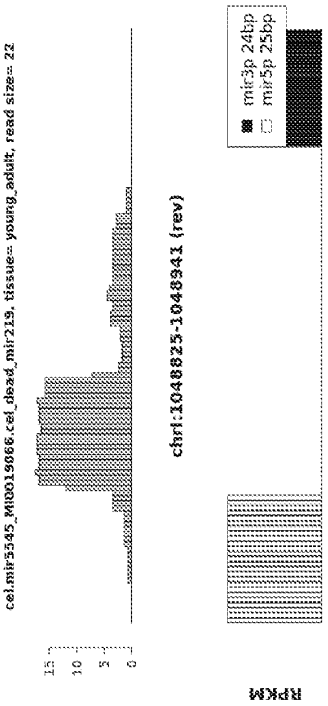


FIG. 13G

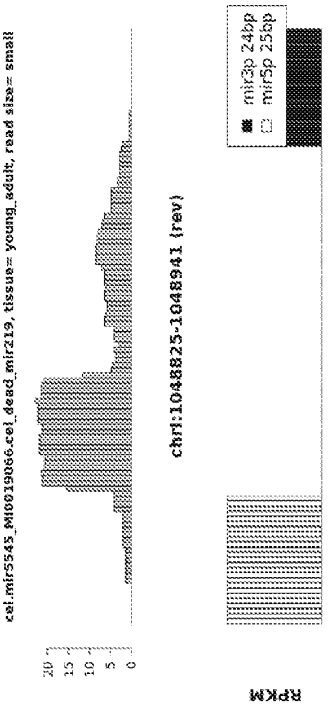


FIG. 13H

cel_dead_mir219 2D structure

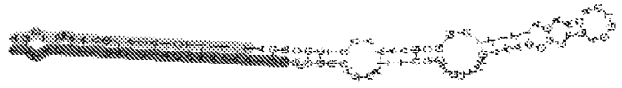


FIG. 14A

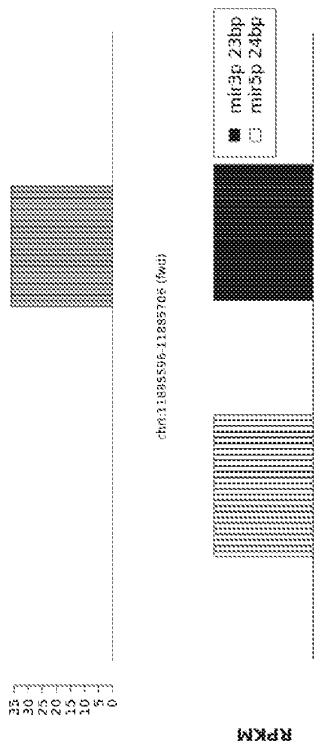


FIG. 14B

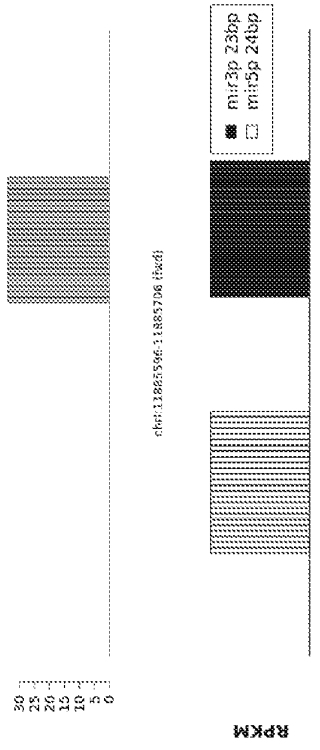


FIG. 14C

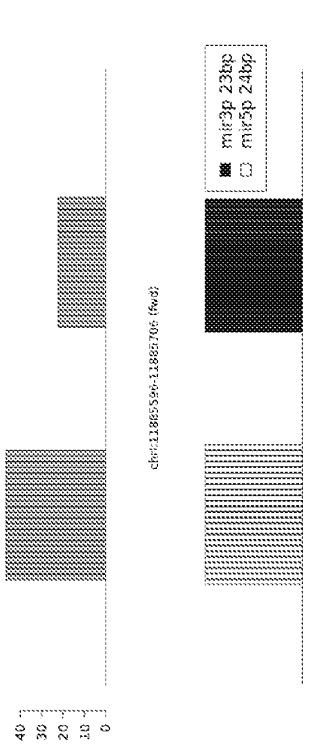


FIG. 14D

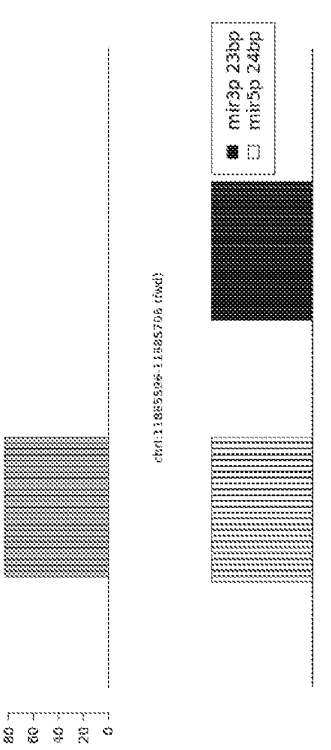


FIG. 14E

cel.mir5545_MI0019066 2D structure

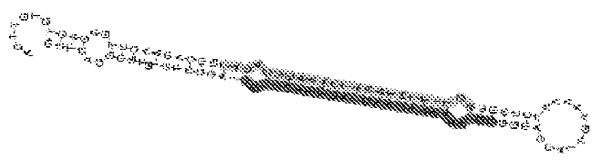


FIG. 14F

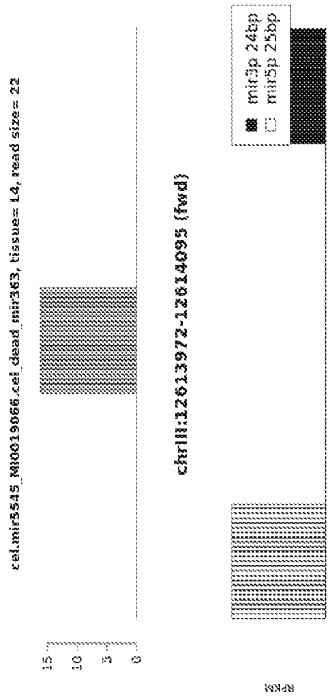


FIG. 14G

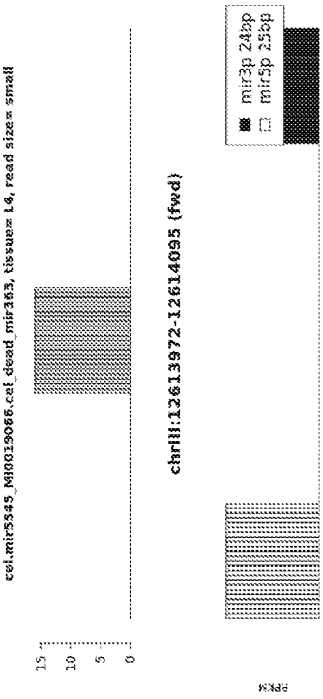


FIG. 14H

cel_dead_mir363 2D structure

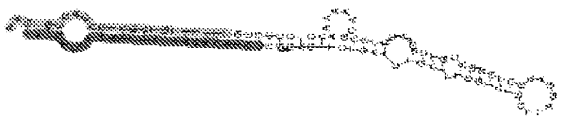


FIG. 15A

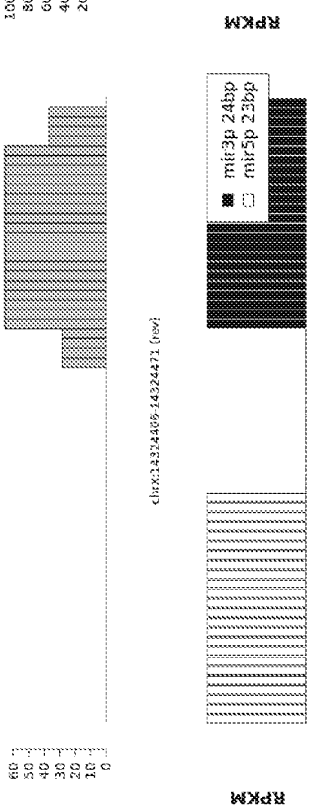


FIG. 15B

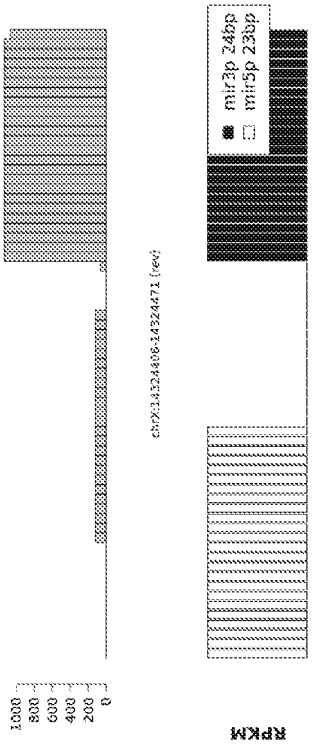


FIG. 15C

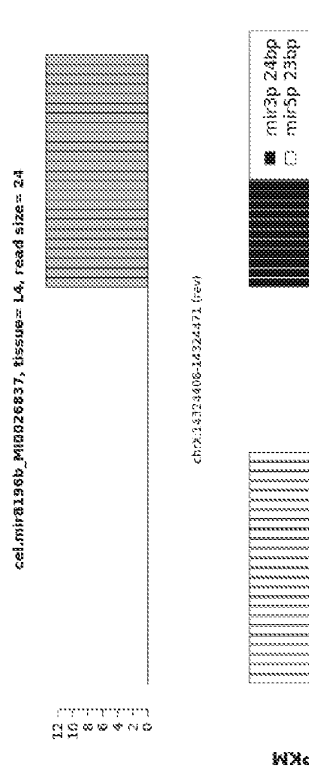


FIG. 15D

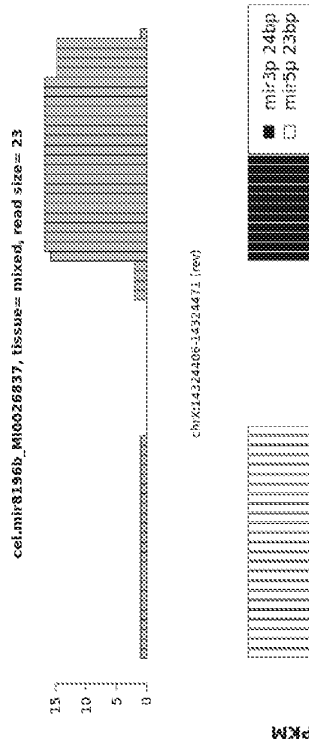


FIG. 15E

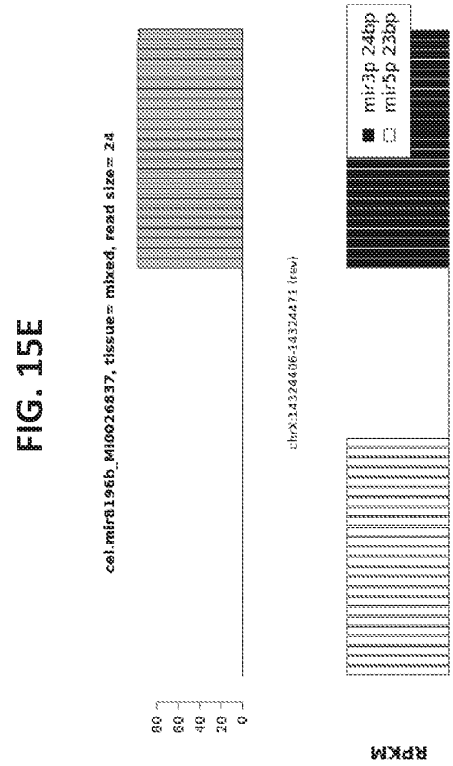


FIG. 15F

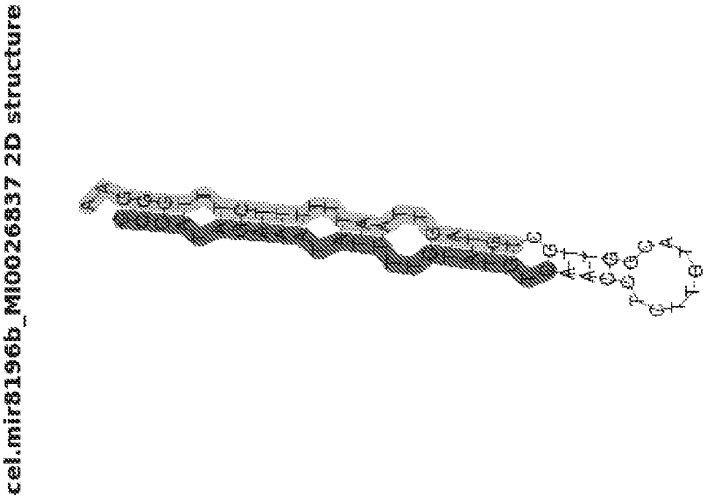


FIG. 15G

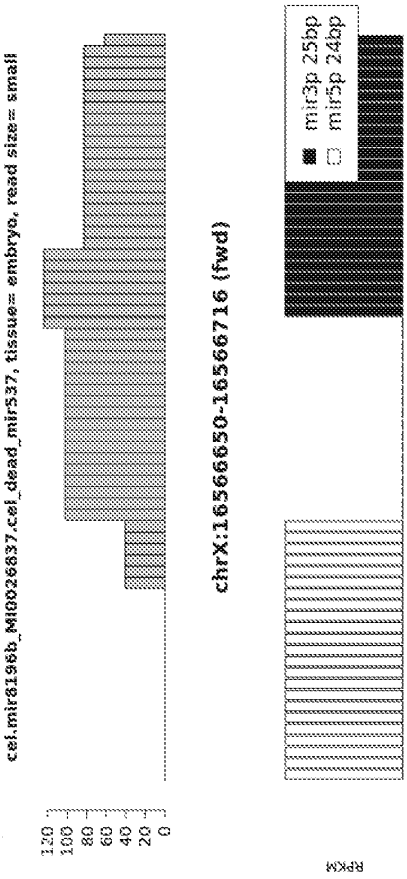


FIG. 15H

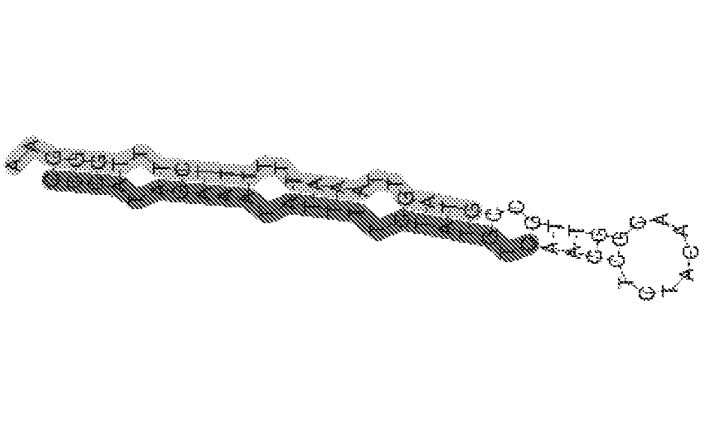


FIG. 16A

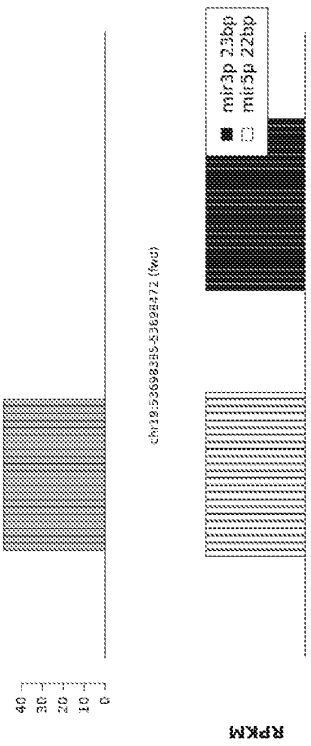


FIG. 16B

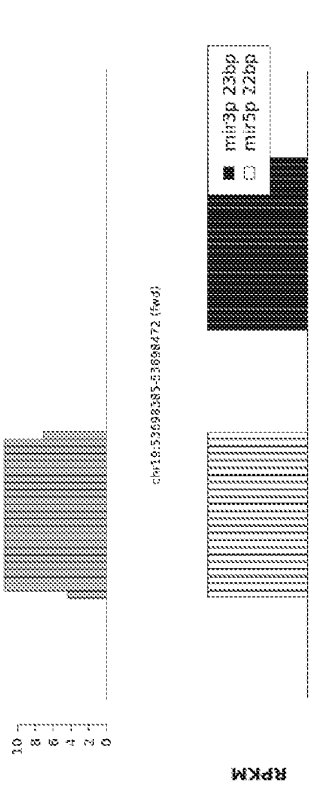


FIG. 16C

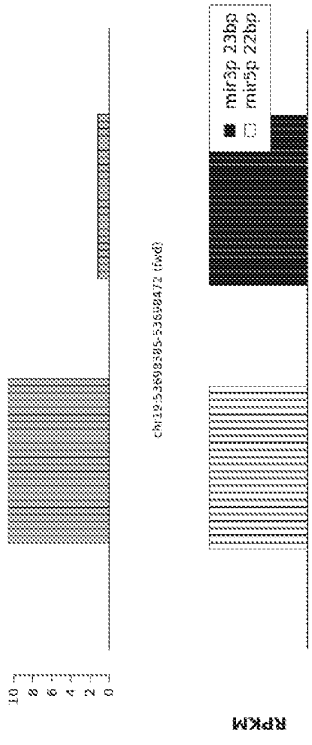


FIG. 16D

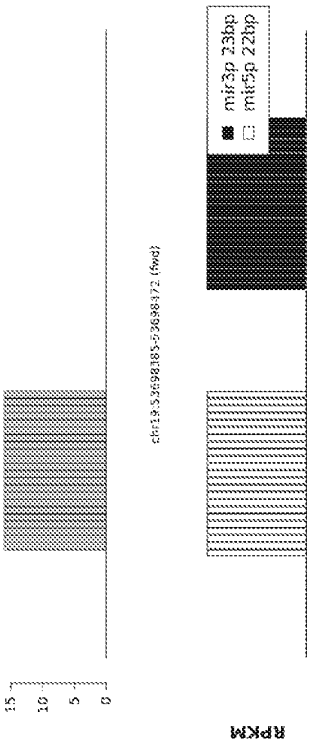


FIG. 16E

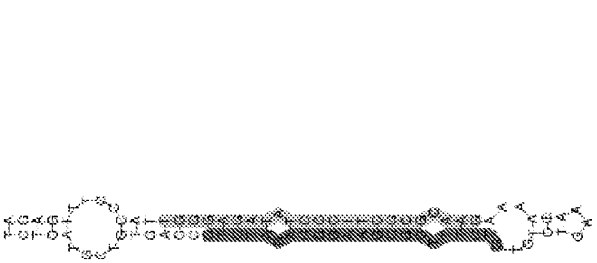


FIG. 16F

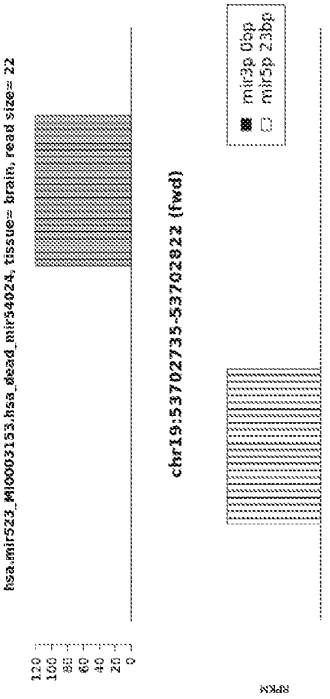


FIG. 16G

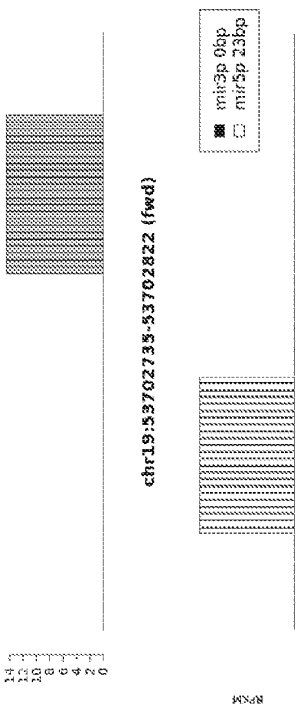


FIG. 16H

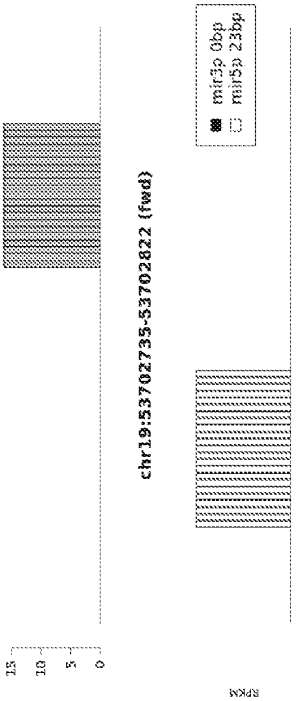


FIG. 16J

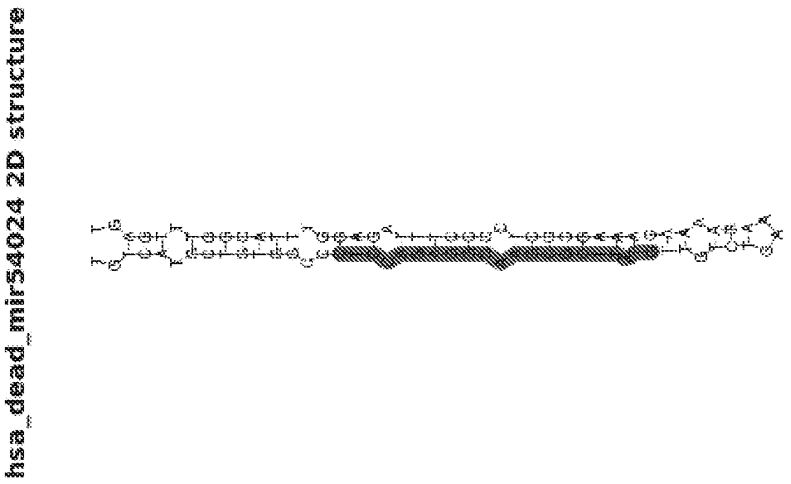


FIG. 16I

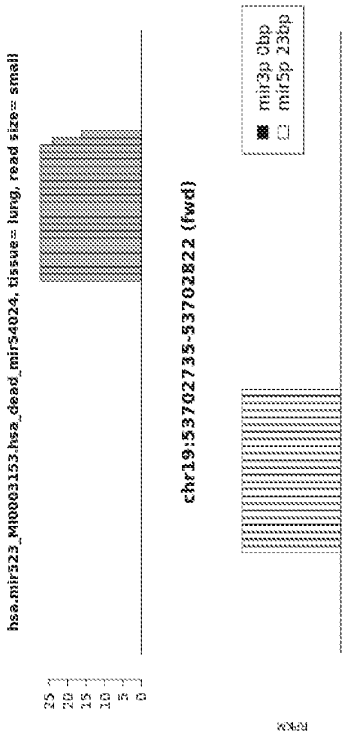


FIG. 17A

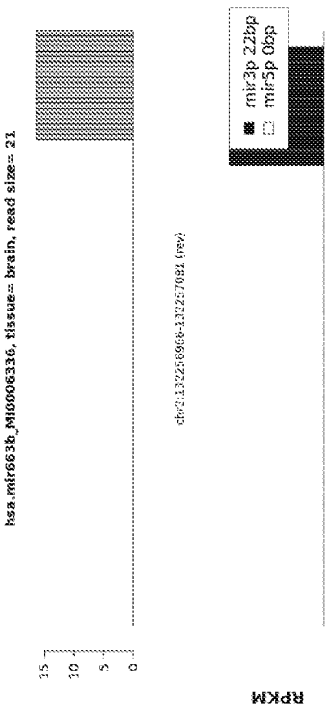


FIG. 17B

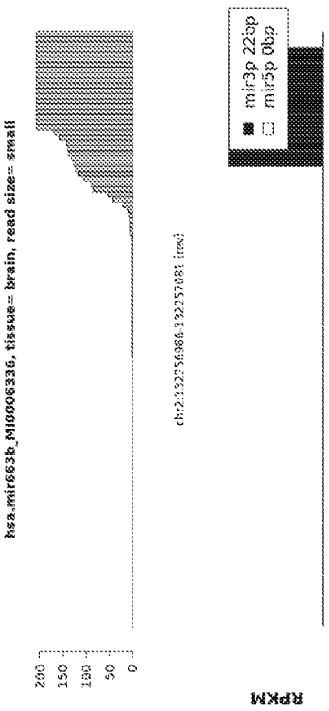


FIG. 17C

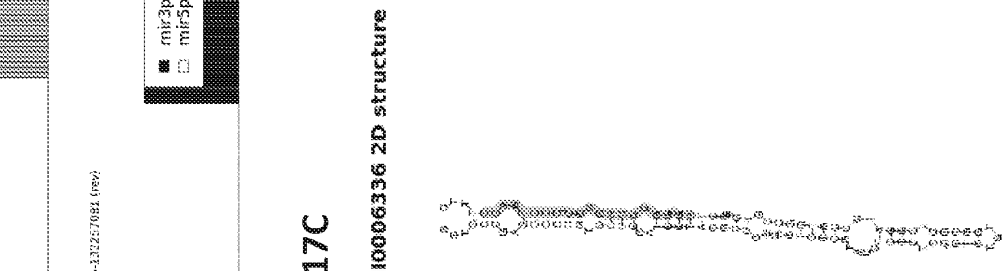


FIG. 17D

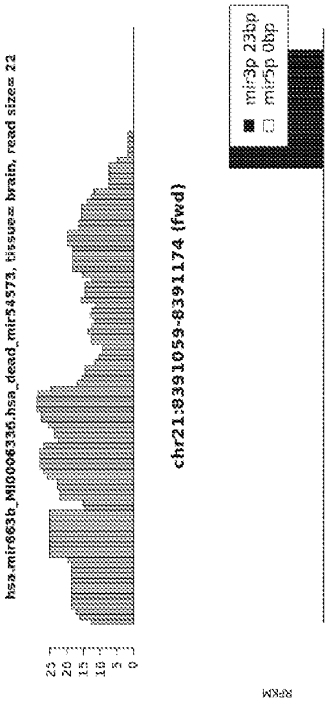


FIG. 17E

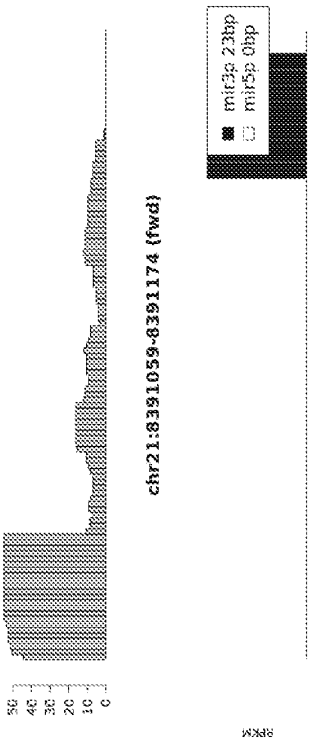


FIG. 17F

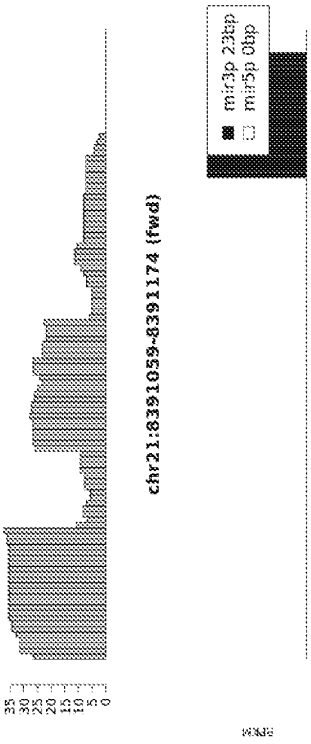


FIG. 17G

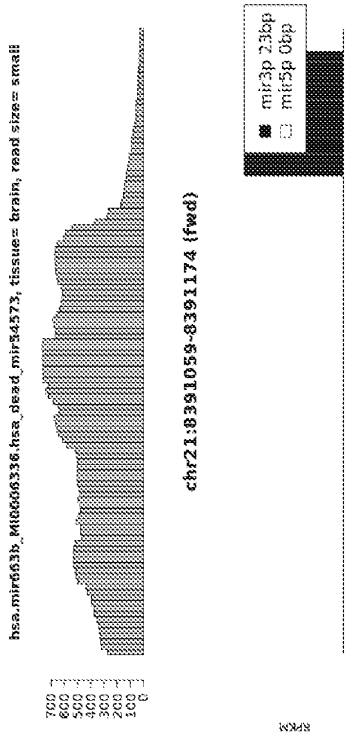


FIG. 17H

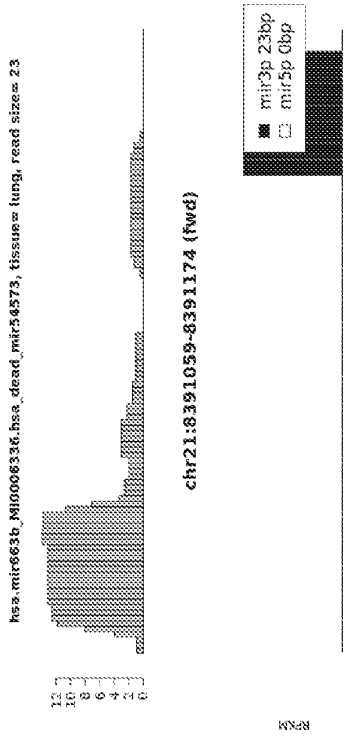


FIG. 17J

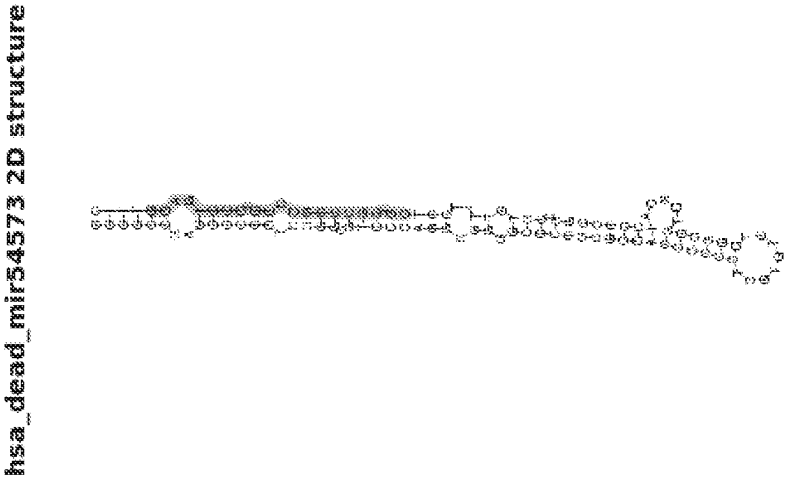


FIG. 17I

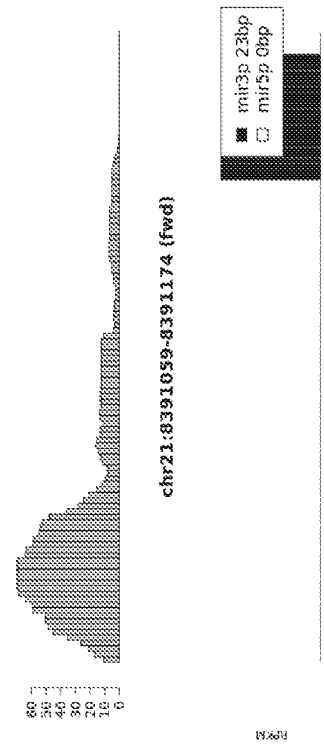


FIG. 18A

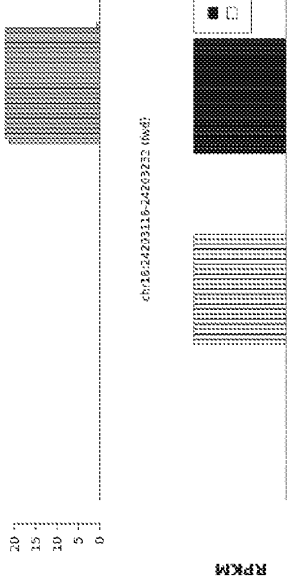


FIG. 18B

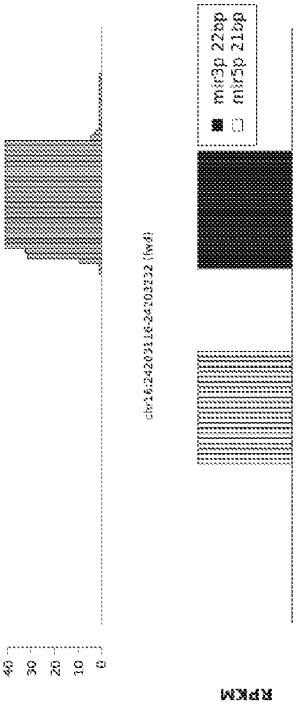


FIG. 18C

hsa.mir1273h_MI0025512 2D structure

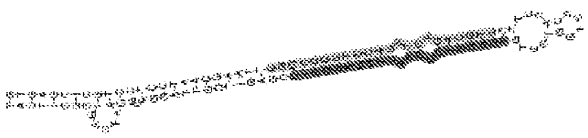


FIG. 18E

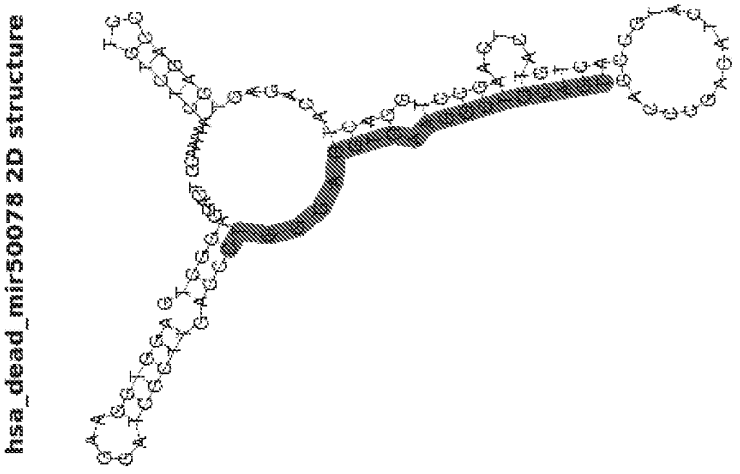


FIG. 18D

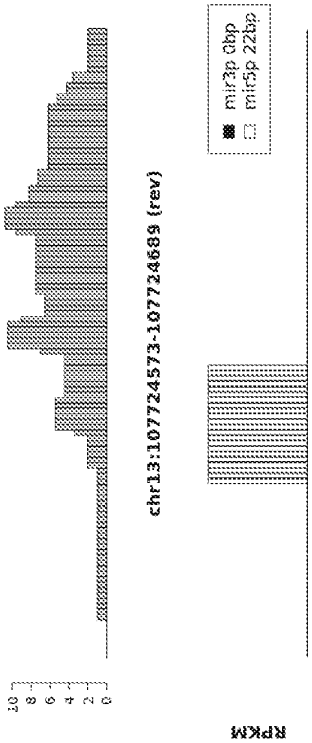


FIG. 19A

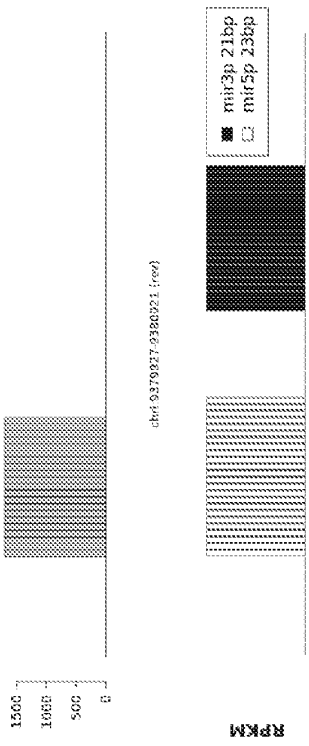


FIG. 19B

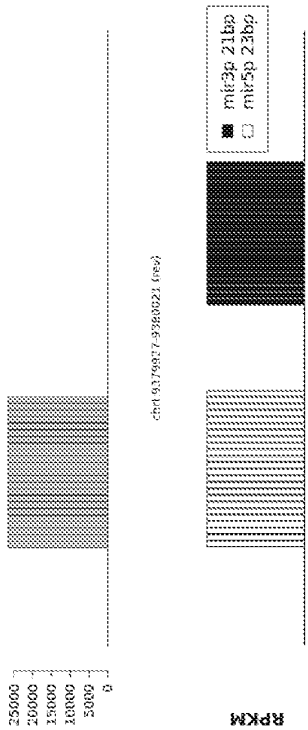


FIG. 19C

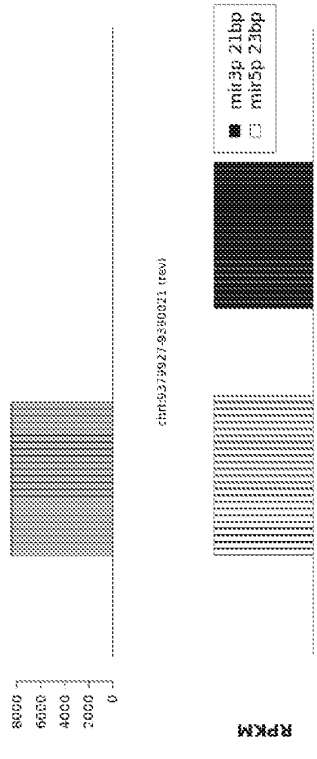


FIG. 19D

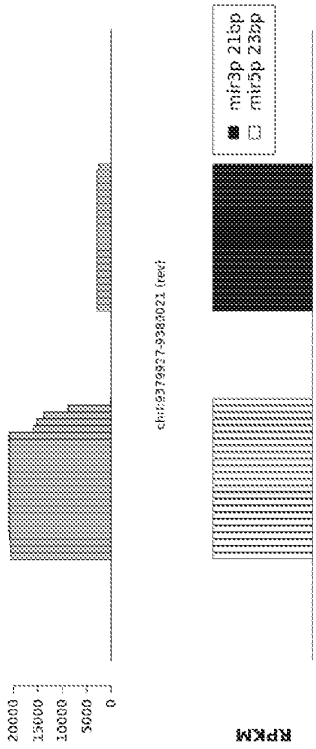


FIG. 19E

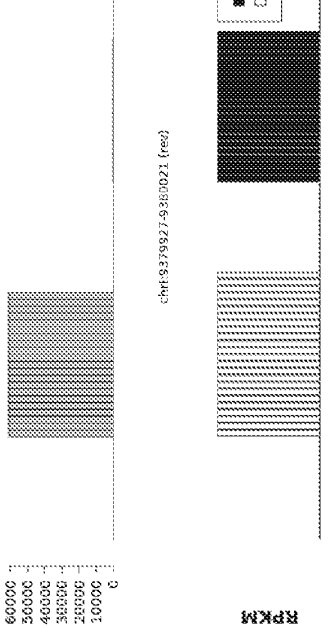


FIG. 19F

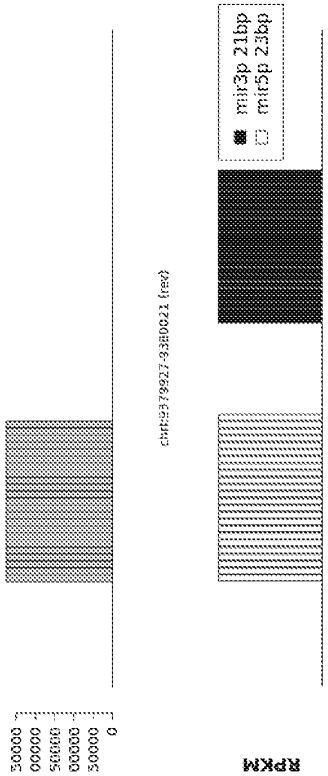


FIG. 19H

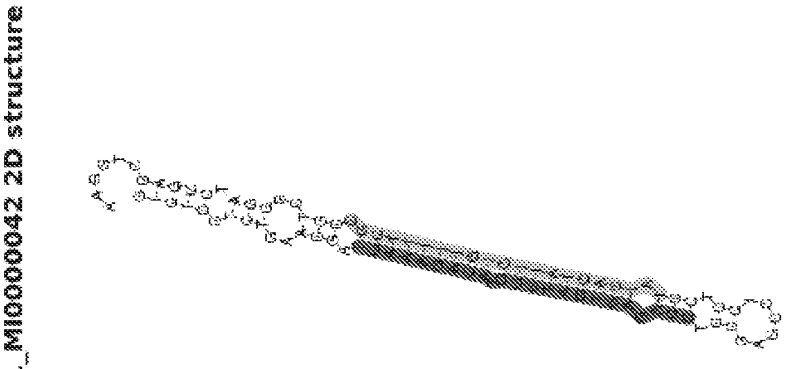


FIG. 19G

