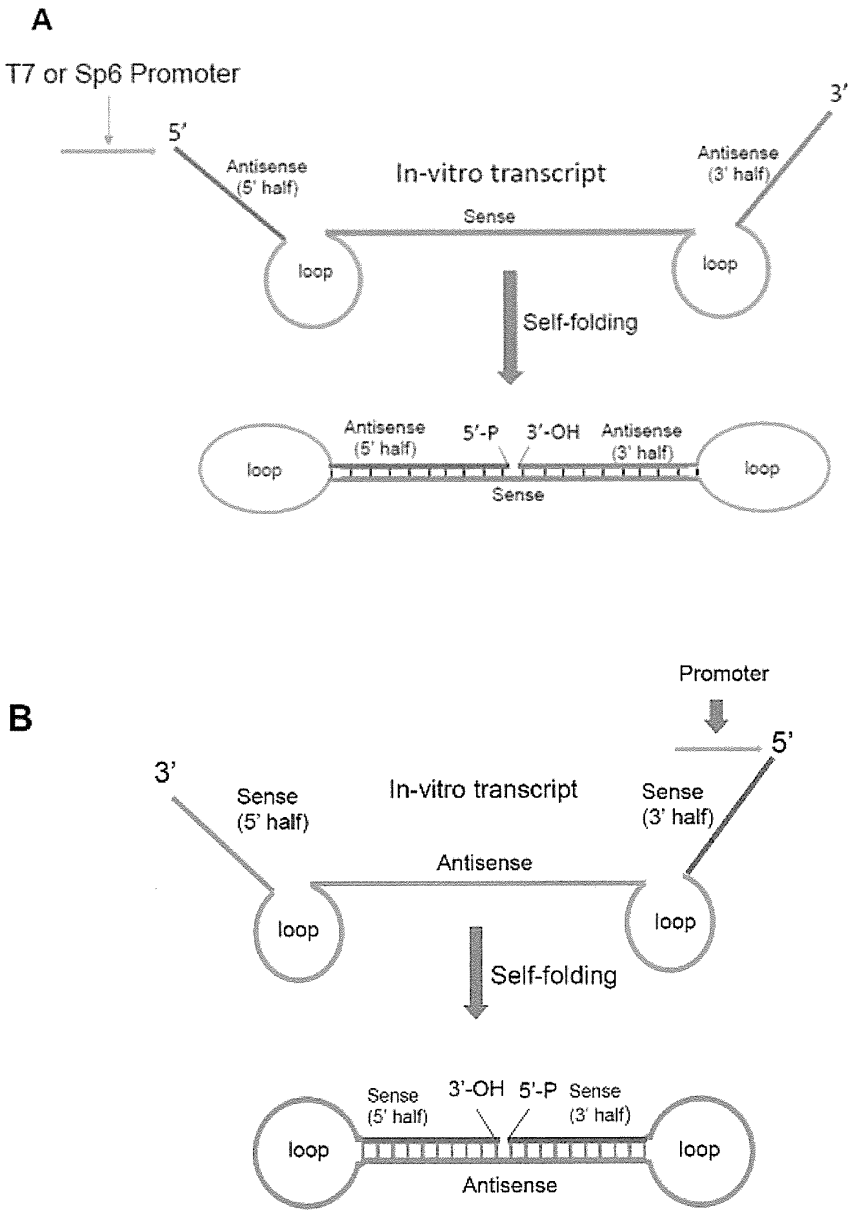

A
T7 or Sp6 Promoter
5'
Antisense
(5' half)
In-vitro transcript
3'
Antisense
(3' half)
Sense
loop
loop
Self-folding
Antisense
(5' half)
5'-P
3'-OH
Antisense
(3' half)
loop
loop
Sense
B
Promoter
5'
3'
Sense
(5' half)
In-vitro transcript
Sense
(3' half)
Antisense
loop
loop
Self-folding
Sense
(5' half)
3'-OH
5'-P
Sense
(3' half)
loop
loop
Antisense

FIGURE 1

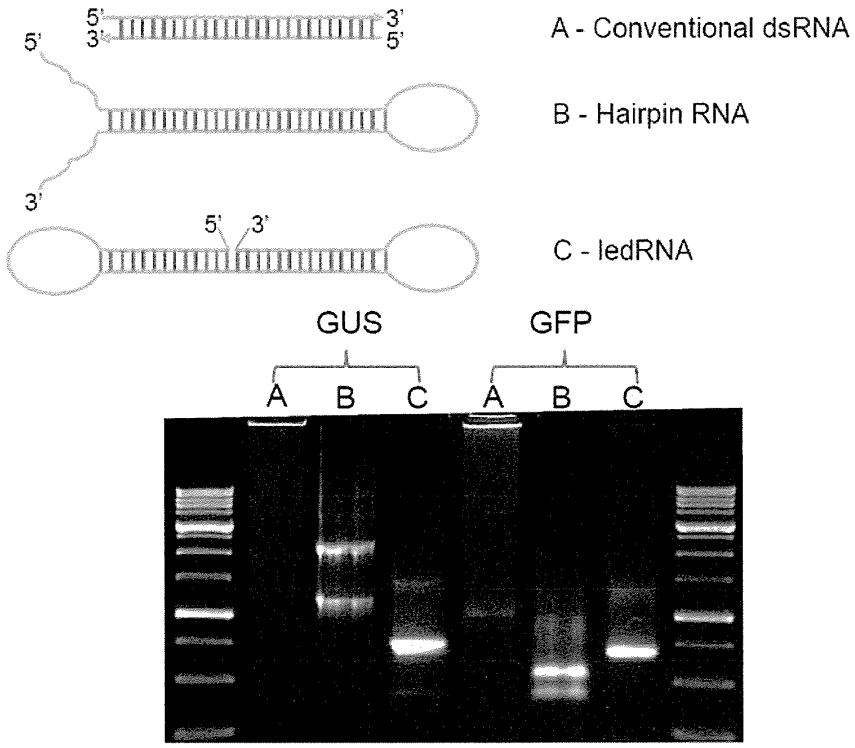

5'
3'
3'
5'
5'
3'
A - Conventional dsRNA
B - Hairpin RNA
5'
3'
C - ledRNA
GUS
GFP
A
B
C
A
B
C

FIGURE 2

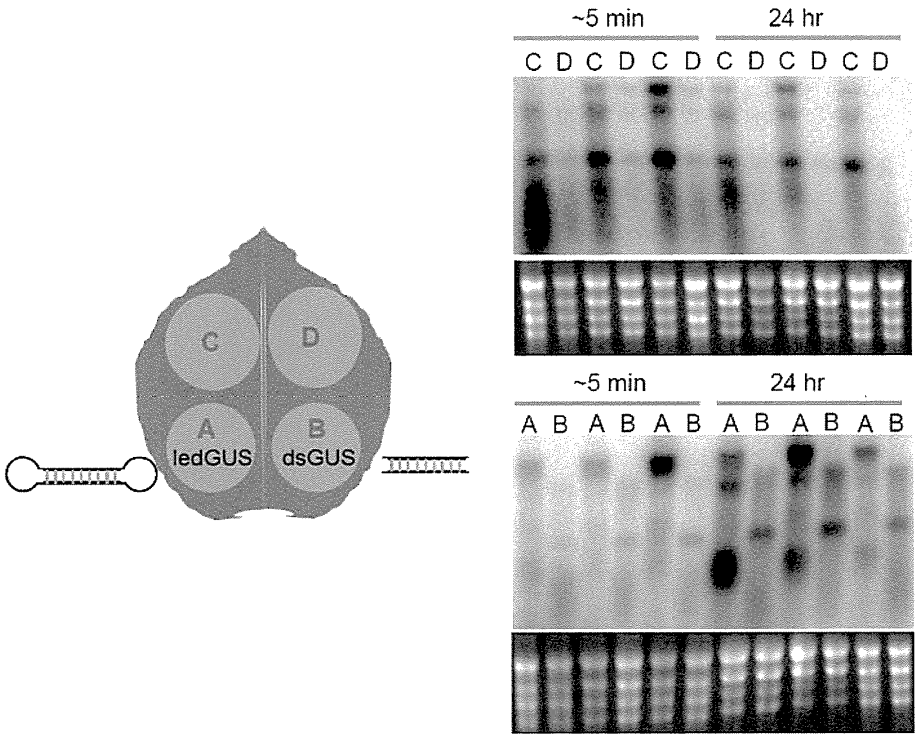

~5 min
24 hr
C D C D C D C D C D C D
C
D
A
B
ledGUS
dsGUS
~5 min
24 hr
A B A B A B A B A B A B

FIGURE 3

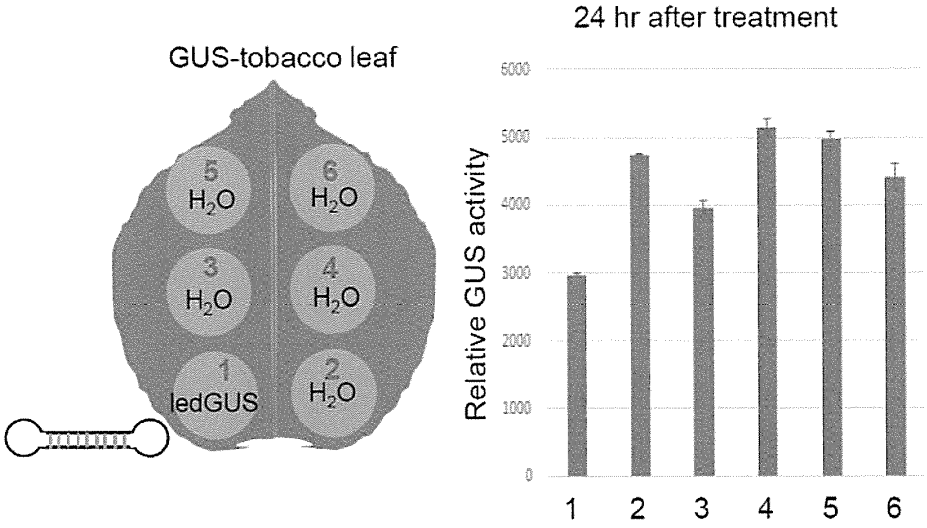

GUS-tobacco leaf
5
H2O
6
H2O
3
H2O
4
H2O
1
ledGUS
2
H2O
24 hr after treatment
Relative GUS activity
6000
5000
4000
3000
2000
1000
0
1
2
3
4
5
6

FIGURE 4

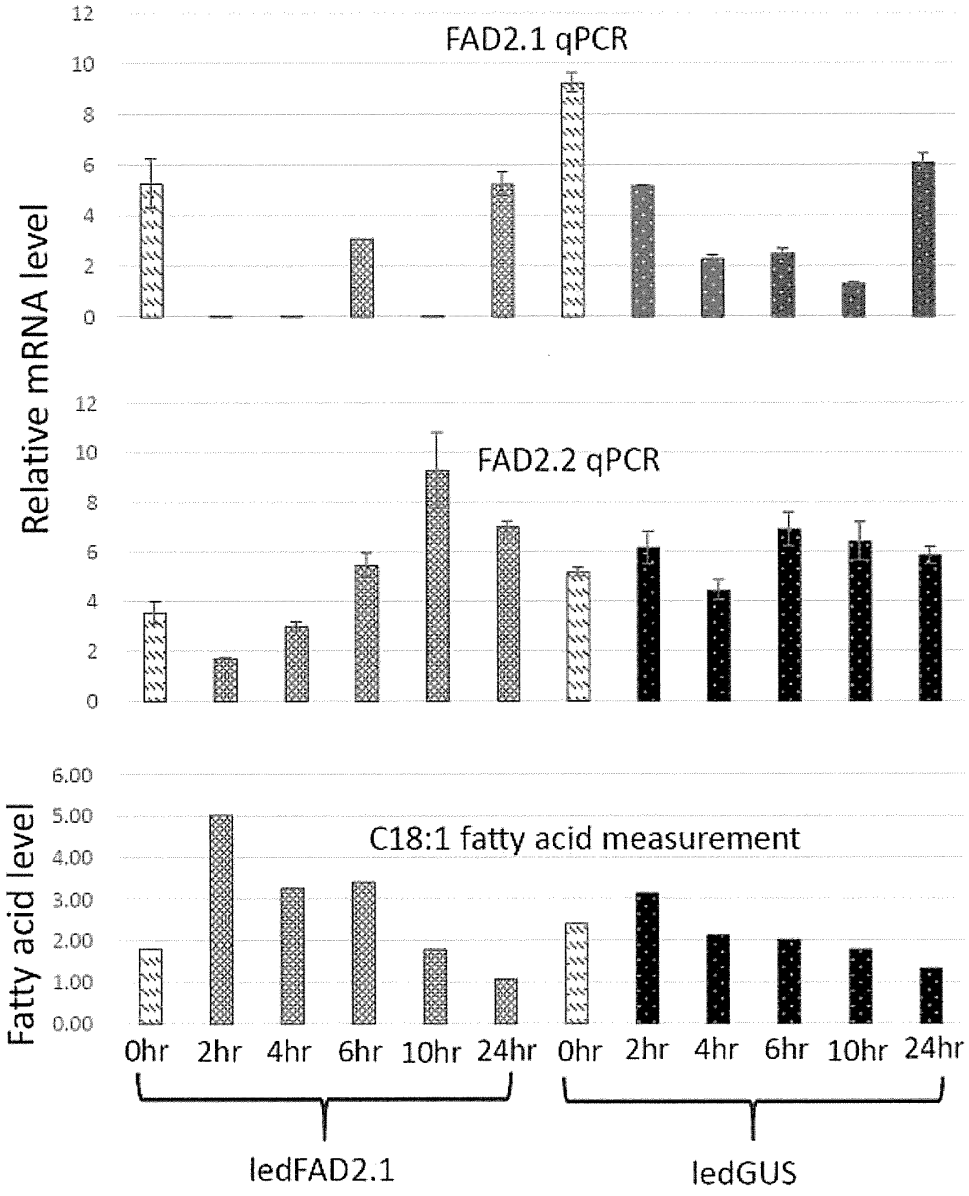

FAD2.1 qPCR
FAD2.2 qPCR
C18:1 fatty acid measurement
Relative mRNA level
Fatty acid level
12
10
8
6
4
2
0
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0hr
2hr
4hr
6hr
10hr
24hr
ledFAD2.1
ledGUS

FIGURE 5

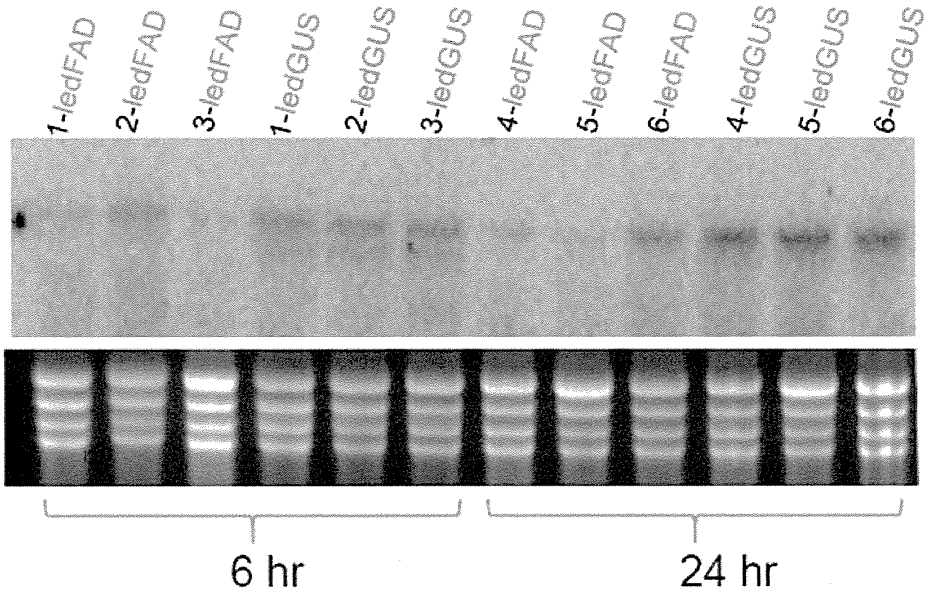

1-ledFAD
2-ledFAD
3-ledFAD
1-ledGUS
2-ledGUS
3-ledGUS
4-ledFAD
5-ledFAD
6-ledFAD
4-ledGUS
5-ledGUS
6-ledGUS
6 hr
24 hr

FIGURE 6

Alignment with WT GUS ORF

```
GUS             GAGTGTGATATCTACCCGCTTCGCGTCGGCATCCGGTCAGTGGCAGTGAAGGGCGAACAG 840
GUS-GU          --------------------TTGTGTTGGTATTTGGTTAGTGGTAGTGAAGGGTGAATAG 40
                                    * * ** ** **  *** ***** ********* *** **

GUS             TTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGGTCGTCATGAAGATGCGGAC 900
GUS-GU          TTTTTGATTAATTATAAATTGTTTTATTTTATTGGTTTTGGTTGTTATGAAGATGTGGAT 100
                **  *******  * ***  *** ** **** *** ****** ** ********* ***

GUS             TTGCGTGGCAAAGGATTCGATAACGTGCTGATGGTGCACGACCACGCATTAATGGACTGG 960
GUS-GU          TTGTGTGGTAAAGGATTTGATAATGTGTTGATGGTGTATGATTATGTATTAATGGATTGG 160
                *** **** ******** ***** *** ******** * **  * * ********* ***

GUS             ATTGGGGCCAACTCCTACCGTACCTCGCATTACCCTTACGCTGAAGAGATGCTCGACTGG 1020
GUS-GU          ATTGGGGTTAATTTTTATTGTATTTTGTATTATTTTTATG-------------------- 200
                *******  ** *  **  ***  * * ****   *** *
```

FIGURE 7

```
GUS             GAGTGTGATATCTACCCGCTTCGCGTCGGCATCCGGTCAGTGGCAGTGAAGGGCGAACAG 840
GUS-4M          --------------------TCGGGTCCGCAACCGCTCACTGGGAGTCAAGCGCGTACAC 40
                                    *** *** ***:*** *** *** *** *** ***:***

GUS             TTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGGTCGTCATGAAGATGCGGAC 900
GUS-4M          TTCGTGAATAAGCACTAACGGTTGTACATTAGTGGGTTTCGTCCTCAAGAACATGGGGAG 100
                *** ***:*** ***:*** *** ***:*** *** *** *** ***:*** *** ***

GUS             TTGCGTGGCAAAGGATTCGATAACGTGCTGATGGTGCACGACCACGCATTAATGGACTGG 960
GUS-4M          TTGGGTGCCAATGGAATCGTTAAGGTGGTGAAGGTCCACCACCTCGCTTTATTGGTCTGC 160
                *** *** ***:***:***:*** *** ***:*** *** ***:***:***:***:***

GUS             ATTGGGGCCAACTCCTACCGTACCTCGCATTACCCTTACGCTGAAGAGATGCTCGACTGG 1020
GUS-4M          ATTCGGGGCAAGTCCAACCCTACGTCGGATTTCCCATACC-------------------- 200
                *** *** *** ***:*** *** *** ***:***:***
```

FIGURE 8

Alignment with WT GUS ORF

```
GUS             GAGTGTGATATCTACCCGCTTCGCGTCGGCATCCGGTCAGTGGCAGTGAAGGGCGAACAG 840
GUS-10M         --------------------TCGCGTCGCGATCCGGTCTCTGGCAGTGTTGGGCGAACTC 40
                                    ********  ********: ********::********:

GUS             TTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGGTCGTCATGAAGATGCGGAC 900
GUS-10M         TTCCTGATATACCACAAAGGGTTCTACTAAACTGGCTTACGTCGTCATCTAGATGCGGTG 100
                ********::********  ********::********: ******** :********:

GUS             TTGCGTGGCAAAGGATTCGATAACGTGCTGATGGTGCACGACCACGCATTAATGGACTGG 960
GUS-10M         TTGCGTGGGTAAGGATTCCTTAACGTGCACATGGTGCAGCACCACGCAAAAATGGACTCC 160
                ******** :******** :********: ********  ********::********

GUS             ATTGGGGCCAACTCCTACCGTACCTCGCATTACCCTTACGCTGAAGAGATGCTCGACTGG 1020
GUS-10M         ATTGGGGCGTACTCCTACGCTACCTCGCTATACCCTTAGC-------------------- 200
                ******** :********  ********::********
```

FIGURE 9

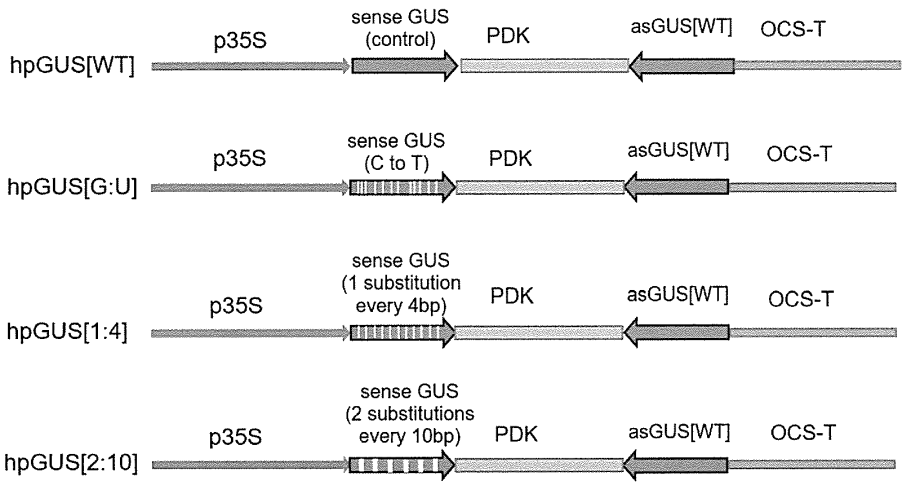

hpGUS[WT]
p35S
sense GUS (control)
PDK
asGUS[WT]
OCS-T
hpGUS[G:U]
p35S
sense GUS (C to T)
PDK
asGUS[WT]
OCS-T
hpGUS[1:4]
p35S
sense GUS (1 substitution every 4bp)
PDK
asGUS[WT]
OCS-T
hpGUS[2:10]
p35S
sense GUS (2 substitutions every 10bp)
PDK
asGUS[WT]
OCS-T

FIGURE 10

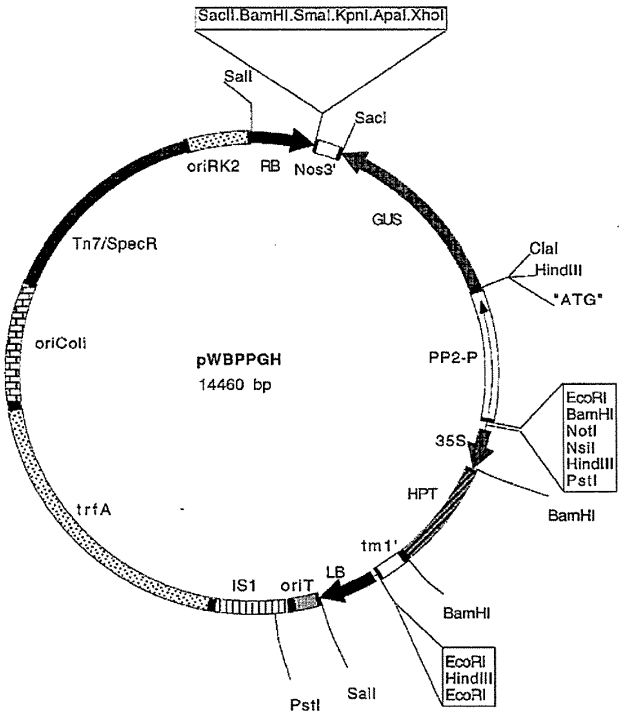

SacII.BamHI.SmaI.KpnI.ApaI.XhoI
SalI
SacI
oriRK2
RB
Nos3'
GUS
Tn7/SpecR
ClaI
HindIII
"ATG"
oriColi
pWBPPGH
14460 bp
PP2-P
EcoRI
BamHI
NotI
NsiI
HindIII
PstI
35S
HPT
BamHI
trfA
tm1'
IS1
oriT
LB
BamHI
EcoRI
HindIII
EcoRI
PstI
SalI

FIGURE 11

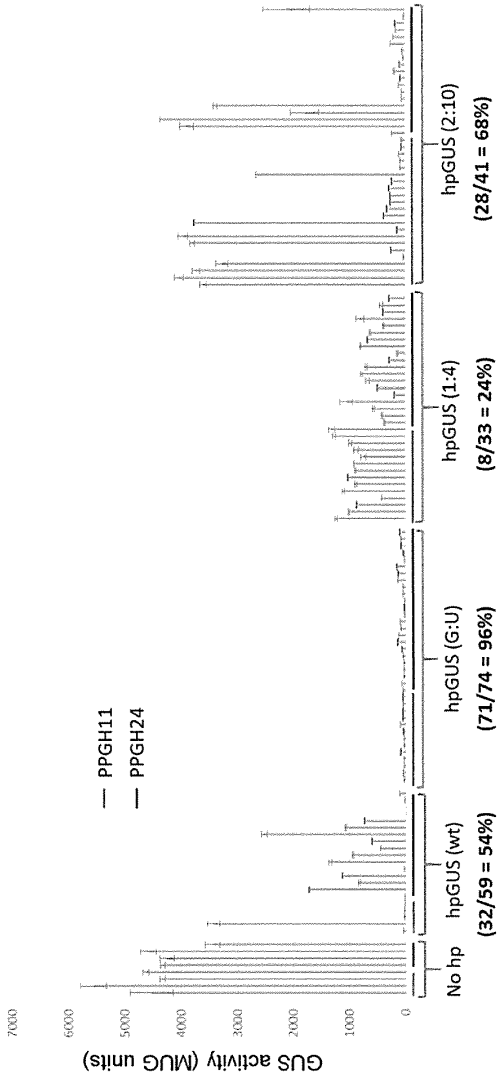

GUS activity (MUG units)
7000
6000
5000
4000
3000
2000
1000
0
PPGH11
PPGH24
No hp
hpGUS (wt)
(32/59 = 54%)
hpGUS (G:U)
(71/74 = 96%)
hpGUS (1:4)
(8/33 = 24%)
hpGUS (2:10)
(28/41 = 68%)

FIGURE 12

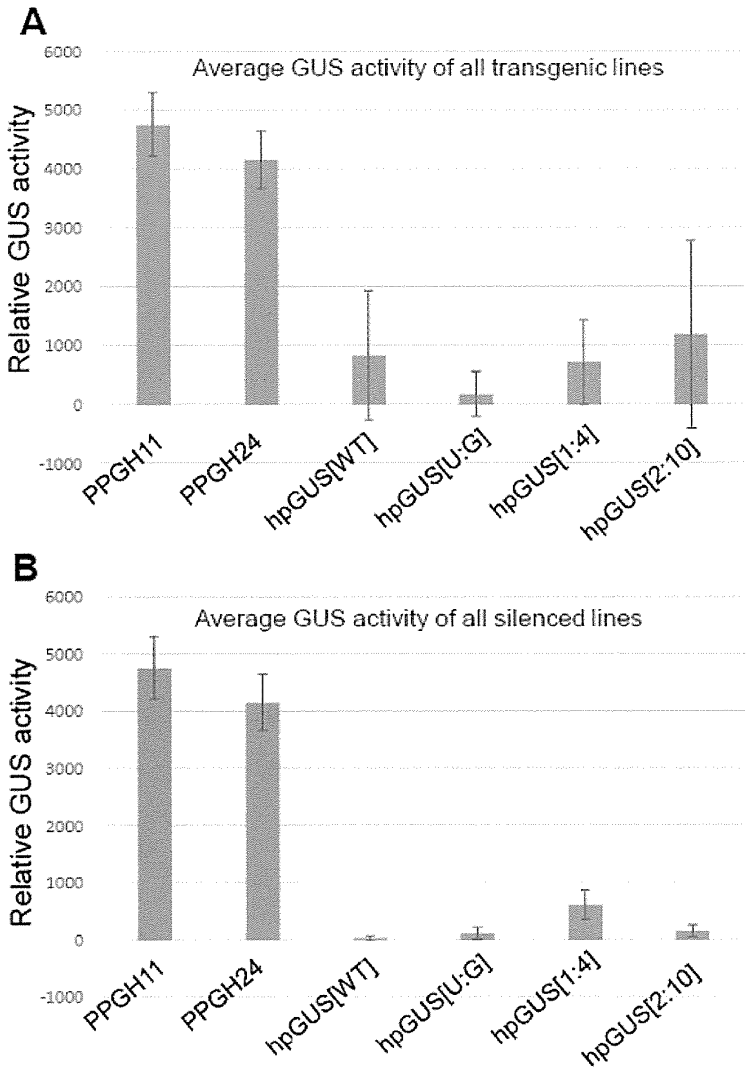

A
Average GUS activity of all transgenic lines
Relative GUS activity
6000
5000
4000
3000
2000
1000
0
-1000
PPGH11
PPGH24
hpGUS[WT]
hpGUS[U:G]
hpGUS[1:4]
hpGUS[2:10]
B
Average GUS activity of all silenced lines
Relative GUS activity
6000
5000
4000
3000
2000
1000
0
-1000
PPGH11
PPGH24
hpGUS[WT]
hpGUS[U:G]
hpGUS[1:4]
hpGUS[2:10]

FIGURE 13

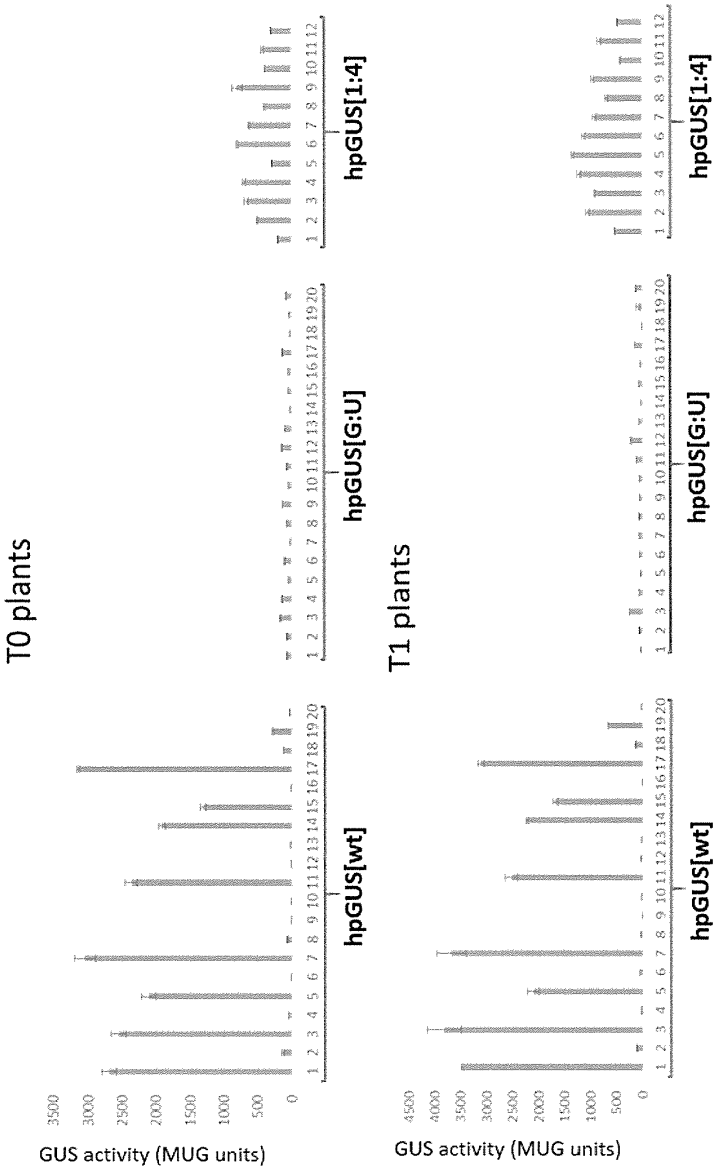

T0 plants
T1 plants
hpGUS[wt]
hpGUS[G:U]
hpGUS[1:4]
GUS activity (MUG units)
GUS activity (MUG units)

FIGURE 14

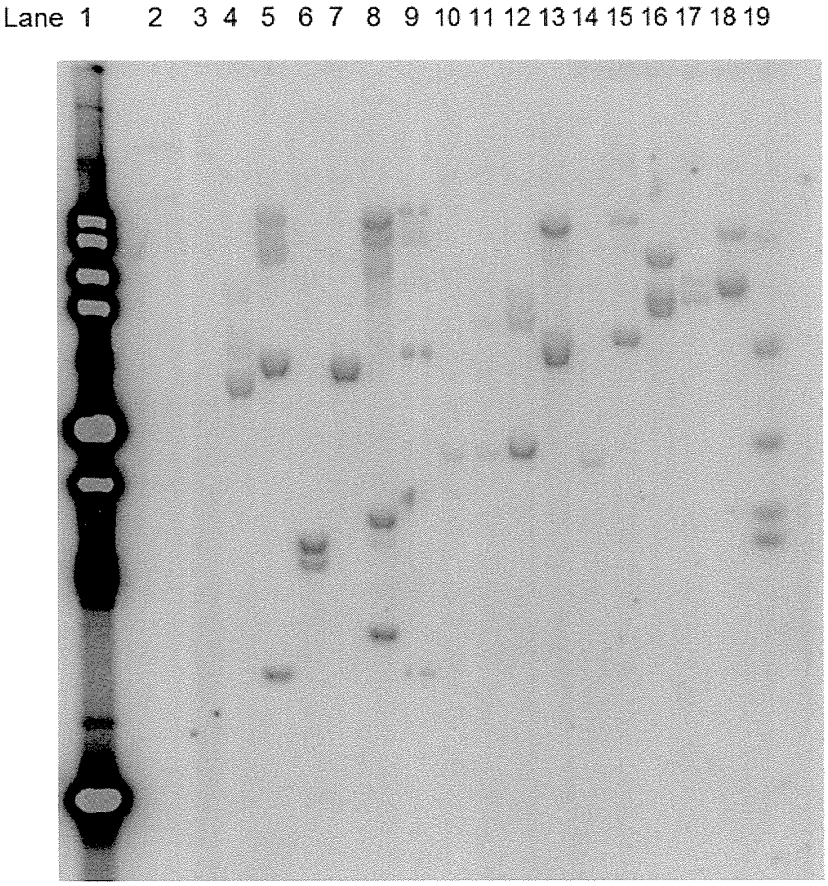


FIGURE 15

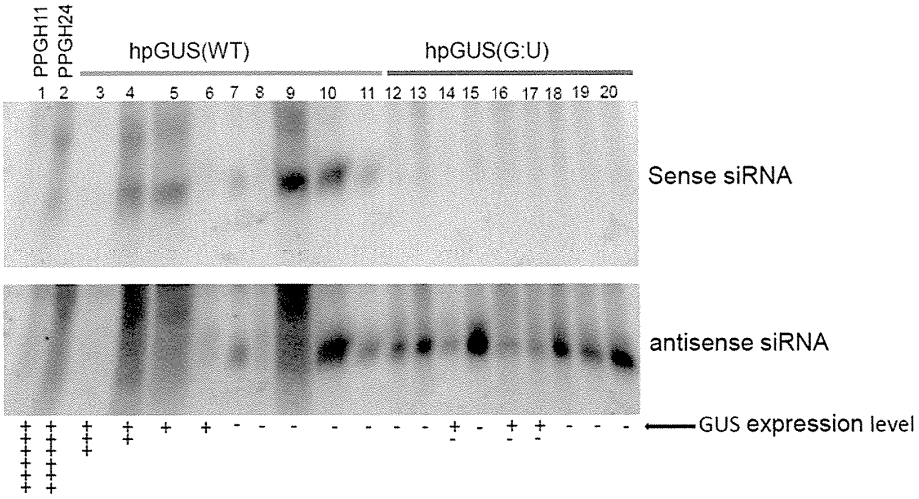

PPGH11
PPGH24
hpGUS(WT)
hpGUS(G:U)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Sense siRNA
antisense siRNA
GUS expression level

FIGURE 16

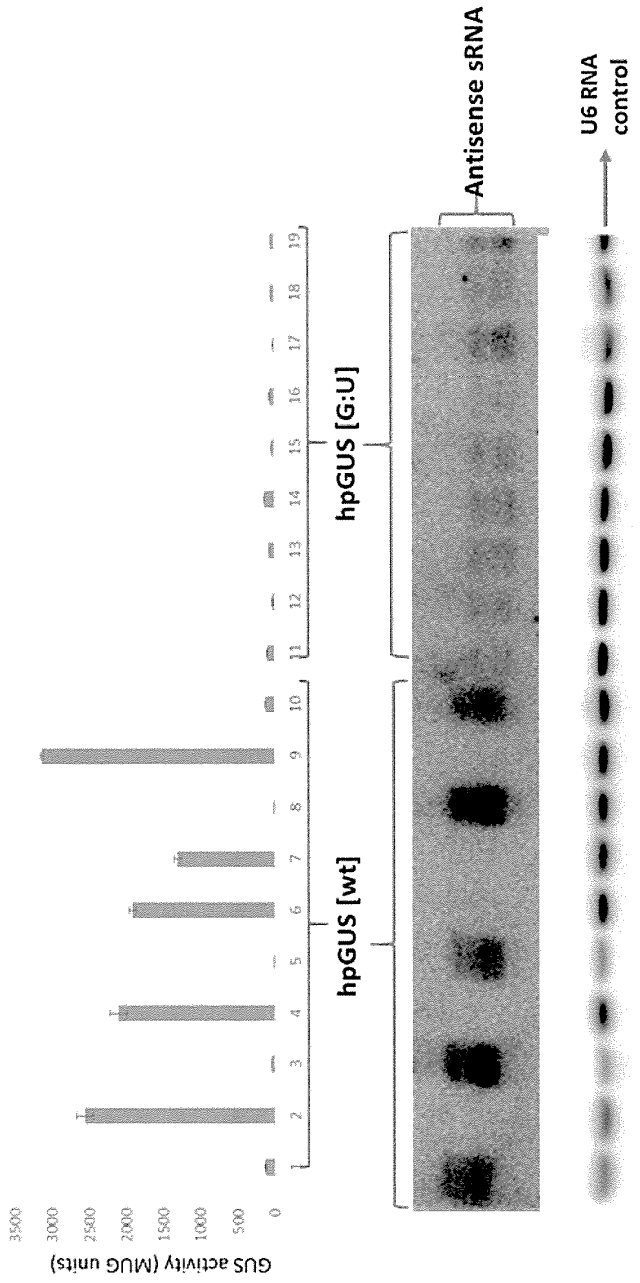

GUS activity (MUG units)
3500
3000
2500
2000
1500
1000
500
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
hpGUS [wt]
hpGUS [G:U]
Antisense sRNA
U6 RNA control

FIGURE 17

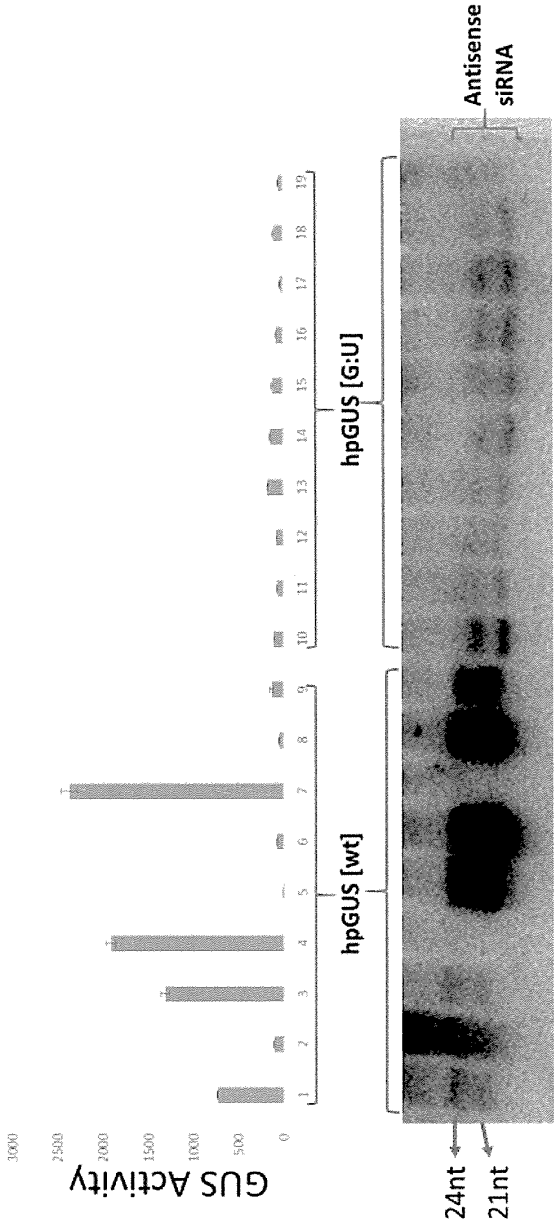

GUS Activity
3000
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
hpGUS [wt]
hpGUS [G:U]
Antisense siRNA
24nt
21nt

FIGURE 18

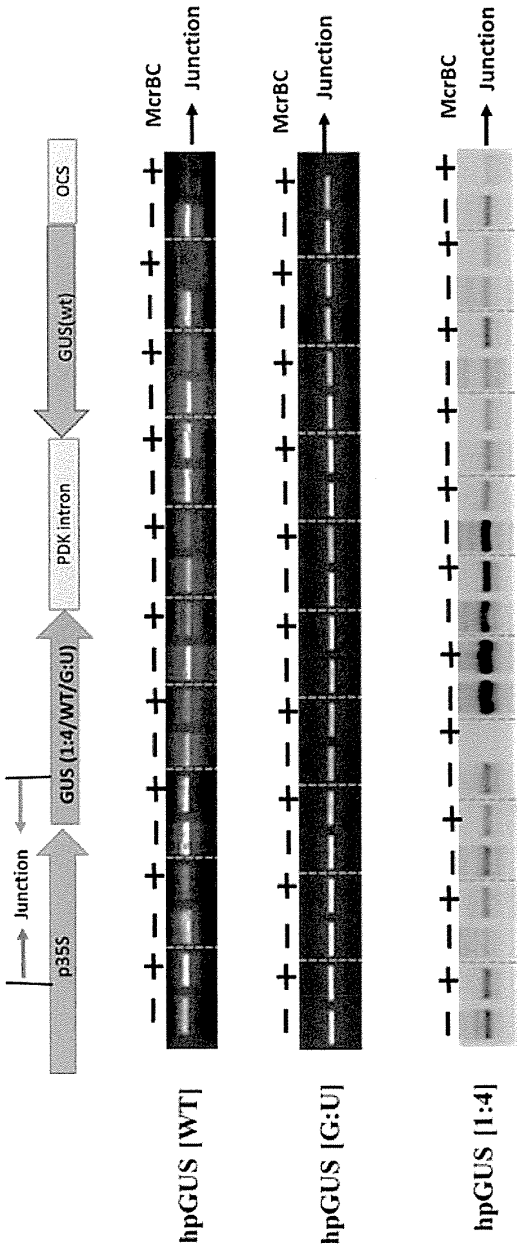

Junction
p35S
GUS (1:4/WT/G:U)
PDK intron
GUS(wt)
OCS
McrBC
Junction
hpGUS [WT]
McrBC
Junction
hpGUS [G:U]
McrBC
Junction
hpGUS [1:4]

FIGURE 19

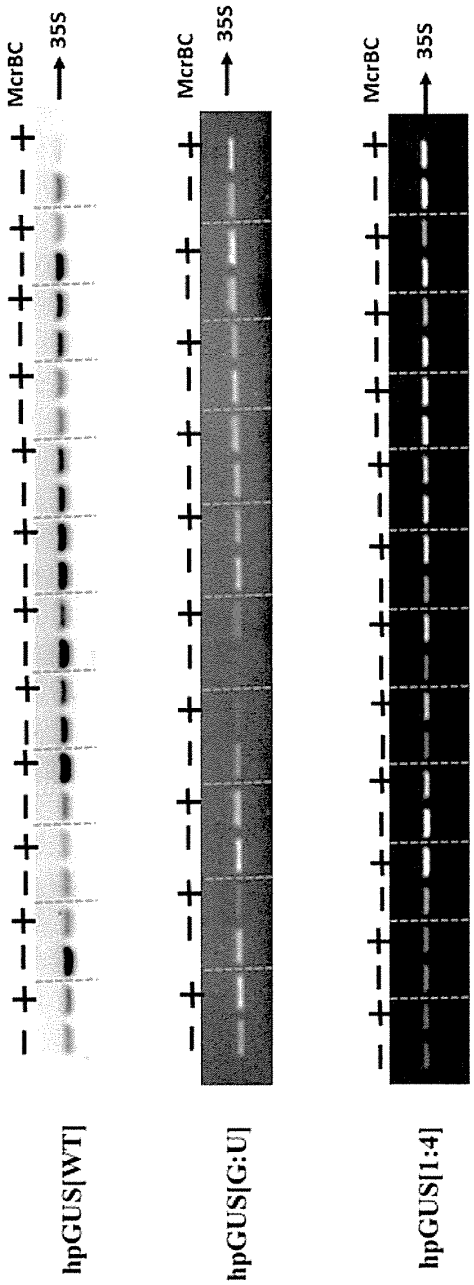


FIGURE 20

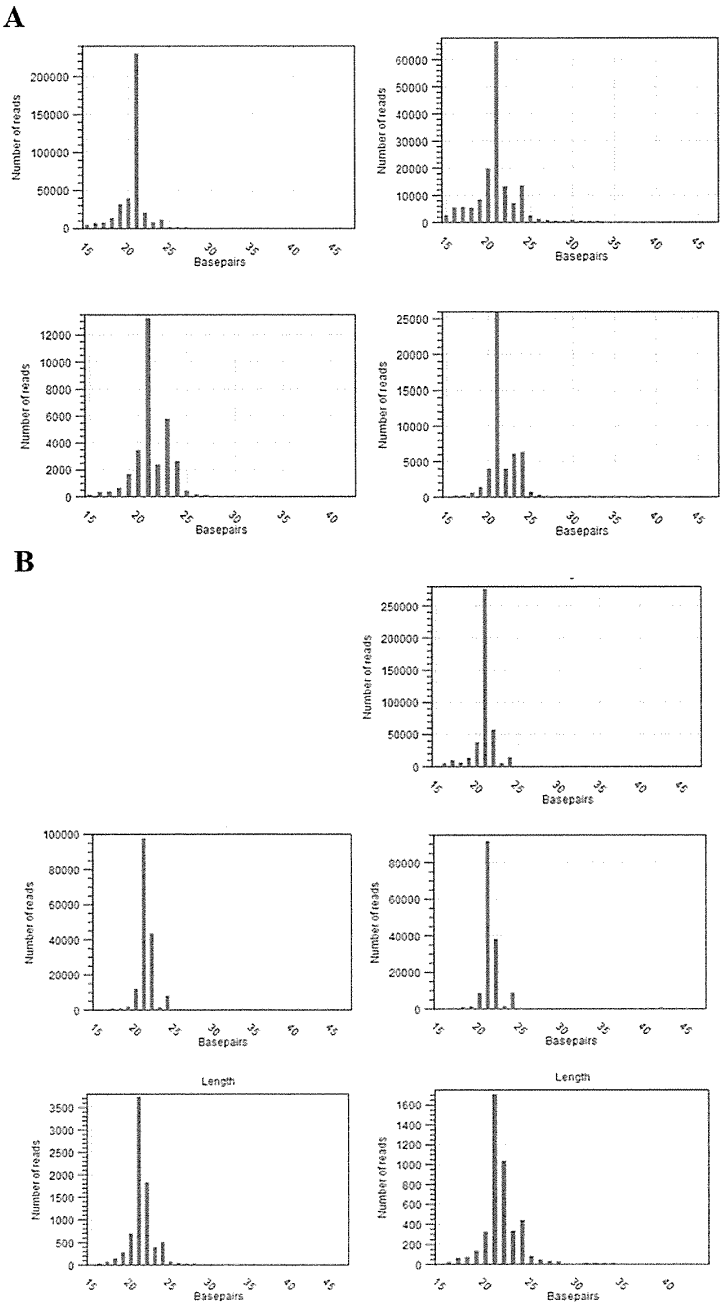

A
Number of reads
Basepairs
B
Number of reads
Basepairs
Length

FIGURE 21

```
EIN2-GU         ----------------------------------------------------GATTTTA
EIN2-cDNA       AGTAATTATATTATCAGATAGATTTAGGATGGAAGCTGAAATTGTGAATGTGAGACCTCA
                                                                   **  * *

EIN2-GU         GTTAGGGTTTATTTAGAGAATGGTTTTTGTTTTATTTTTTGTTTTTTTGGTTTTTGTTGG
EIN2-cDNA       GCTAGGGTTTATCCAGAGAATGGTTCCTGCTCTACTTCCTGTCCTTTTGGTTTCTGTCGG
                * **********  ***********  ** * ** **  ***  ********* *** **

EIN2-GU         ATATATTGATTTTGGGAAATGGGTTGTAAATATTGAAGGAGGTGTTTGTTTTGGGTATGA
EIN2-cDNA       ATATATTGATCCCGGGAAATGGGTTGCAAATATCGAAGGAGGTGCTCGTTTCGGGTATGA
                **********   ************* ****** ********** * **** ********

EIN2-GU         TTTGGTGGTAATTATTTTGTTTTTTAATTTTGTTGTTATTTTATGTTAATATGTTGTAGT
EIN2-cDNA       CTTGGTGGCAATTACTCTGCTTTTCAATTTTGCCGCCATCTTATGCCAATATGTTGCAGC
                 ******* ***** * ** **** *******  *  ** *****  ********* **

EIN2-GU         TTGTATAAGTGTT----------------------------------------------
EIN2-cDNA       TCGCATAAGCGTTGTGACTGGTAAACACTTGGCTCAGATCTGCAATGAAGAATATGACAA
                * * ***** ***
```

FIGURE 22

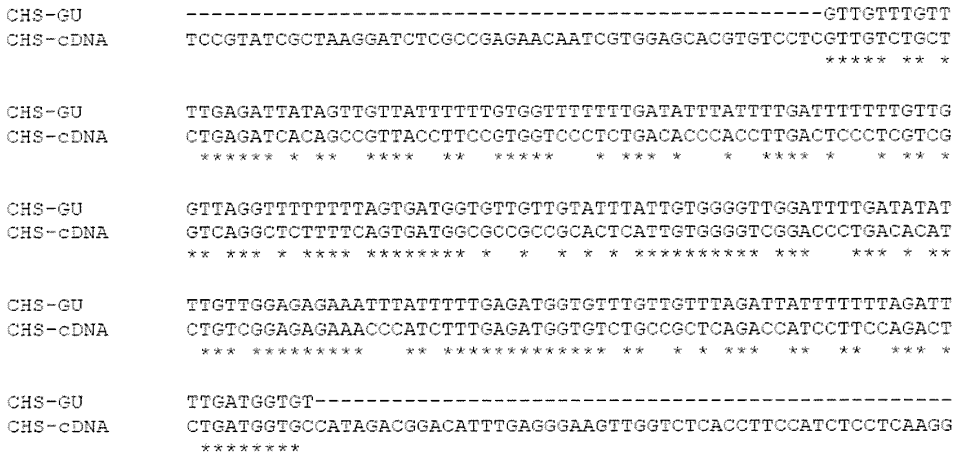

CHS-GU ----------GTTGTTTGTT
CHS-cDNA TCCGTATCGCTAAGGATCTCGCCGAGAACAATCGTGGAGCACGTGTCCTCGTTGTCTGCT
*****
CHS-GU TTGAGATTATAGTTGTTATTTTTTGTGGTTTTTTTGATATTTATTTTGATTTTTTTGTTG
CHS-cDNA CTGAGATCACAGCCGTTACCTTCCGTGGTCCCTCTGACACCCACCTTGACTCCCTCGTCG
CHS-GU GTTAGGTTTTTTTTAGTGATGGTGTTGTTGTATTTATTGTGGGGTTGGATTTTGATATAT
CHS-cDNA GTCAGGCTCTTTTCAGTGATGGCGCCGCCGCACTCATTGTGGGGTCGGACCCTGACACAT
CHS-GU TTGTTGGAGAGAAATTTATTTTTGAGATGGTGTTTGTTGTTTAGATTATTTTTTTAGATT
CHS-cDNA CTGTCGGAGAGAAACCCATCTTTGAGATGGTGTCTGCCGCTCAGACCATCCTTCCAGACT
CHS-GU TTGATGGTGT----------
CHS-cDNA CTGATGGTGCCATAGACGGACATTTGAGGGAAGTTGGTCTCACCTTCCATCTCCTCAAGG
****

FIGURE 23

Antisense EIN2-GU align with antisense EIN2 cDNA

```
As-EIN2-GU      ------------------------------------AATGTTTATGTGAGTTGTAATA
asEIN2-cDNA     TCTTCATTGCAGATCTGAGCCAAGTGTTTACCAGTCACAACGCTTATGCGAGCTGCAACA
                                                    ** * ***** *** ** ** *

As-EIN2-GU      TATTGGTATAAGATGGTGGTAAAATTGAAAAGTAGAGTAATTGTTATTAAGTTATATTTG
asEIN2-cDNA     TATTGGCATAAGATGGCGGCAAAATTGAAAAGCAGAGTAATTGCCACCAAGTCATACCCG
                ****** ********** ** ************ ***********  *  **** ***   *

As-EIN2-GU      AAATGAGTATTTTTTTTGATATTTGTAATTTATTTTTTGGGATTAATATATTTGATAGAA
asEIN2-cDNA     AAACGAGCACCTCCTTCGATATTTGCAACCCATTTCCCGGGATCAATATATCCGACAGAA
                *** *** *  *  ** ********* **   ****   ***** *******  ** ****

As-EIN2-GU      ATTAAAAGGATAGGAAGTAGAGTAGGAATTATTTTTTGGATAAATTTTAGTTGAGGTT--
asEIN2-cDNA     ACCAAAAGGACAGGAAGTAGAGCAGGAACCATTCTCTGGATAAACCCTAGCTGAGGTCTC
                *  ******* *********** *****  *** * ********   *** ******
```

FIGURE 24

Antisense CHS-GU align with antisense CHS cDNA

```
As-CHS-GU       ----------------------------------------GTATTATTAGAGTTTGGAA
asCHS-cDNA      GATGGAAGGTGAGACCAACTTCCCTCAAATGTCCGTCTATGGCACCATCAGAGTCTGGAA
                                                       * *  ** ***** *****

As-CHS-GU       GGATGGTTTGAGTGGTAGATATTATTTTAAAGATGGGTTTTTTTTTGATAGATGTGTTAG
asCHS-cDNA      GGATGGTCTGAGCGGCAGACACCATCTCAAAGATGGGTTTCTCTCCGACAGATGTGTCAG
                ******* **** ** *** *  ** * ************ * *  ** ******** **

As-CHS-GU       GGTTTGATTTTATAATGAGTGTGGTGGTGTTATTATTGAAAAGAGTTTGATTGATGAGGG
asCHS-cDNA      GGTCCGACCCCACAATGAGTGCGGCGGCGCCATCACTGAAAAGAGCCTGACCGACGAGGG
                ***  **    * ******** ** ** *  ** * **********  ***  ** *****

As-CHS-GU       AGTTAAGGTGGGTGTTAGAGGGATTATGGAAGGTAATGGTTGTGATTTTAGAGTAGATAA
asCHS-cDNA      AGTCAAGGTGGGTGTCAGAGGGACCACGGAAGGTAACGGCTGTGATCTCAGAGCAGACAA
                *** *********** *******  * ********** ** ****** * **** *** **
```

FIGURE 25

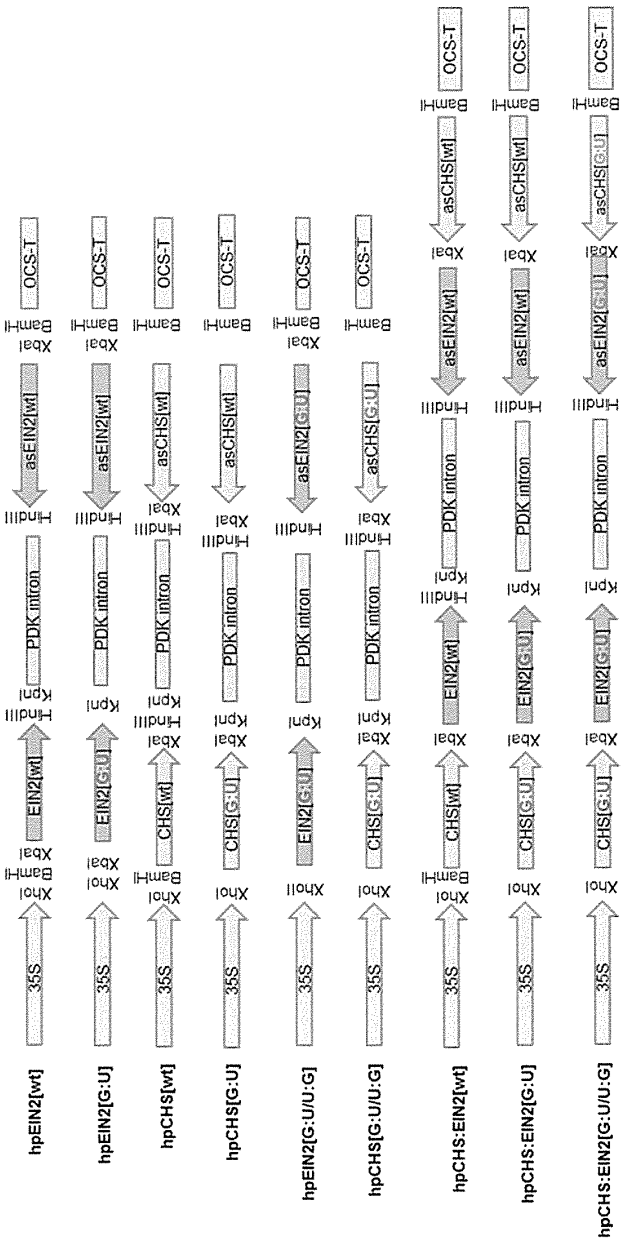

hpEIN2[wt]
35S
XhoI
BamHI
XbaI
EIN2[wt]
HindIII
KpnI
PDK intron
XbaI
BamHI
OCS-T
hpEIN2[G:U]
35S
XhoI
XhoI
XbaI
EIN2[G:U]
KpnI
PDK intron
HindIII
XbaI
BamHI
OCS-T
hpCHS[wt]
35S
XhoI
BamHI
CHS[wt]
XbaI
KpnI
PDK intron
HindIII
XbaI
BamHI
OCS-T
hpCHS[G:U]
35S
XhoI
CHS[G:U]
XbaI
KpnI
PDK intron
HindIII
XbaI
BamHI
OCS-T
hpEIN2[G:U/U:G]
35S
XhoII
EIN2[G:U]
KpnI
PDK intron
HindIII
XbaI
BamHI
OCS-T
hpCHS[G:U/U:G]
35S
XhoI
CHS[G:U]
XbaI
KpnI
PDK intron
HindIII
XbaI
BamHI
OCS-T
hpCHS:EIN2[wt]
35S
XhoI
BamHI
CHS[wt]
XbaI
KpnI
EIN2[wt]
HindIII
PDK intron
XbaI
BamHI
asCHS[wt]
XbaI
HindIII
asEIN2[wt]
BamHI
OCS-T
hpCHS:EIN2[G:U]
35S
XhoI
CHS[G:U]
XbaI
KpnI
EIN2[G:U]
HindIII
PDK intron
XbaI
BamHI
asCHS[wt]
XbaI
HindIII
asEIN2[wt]
BamHI
OCS-T
hpCHS:EIN2[G:U/U:G]
35S
XhoI
CHS[G:U]
XbaI
KpnI
EIN2[G:U]
HindIII
PDK intron
XbaI
BamHI
asCHS[G:U]
XbaI
HindIII
asEIN2[G:U]
BamHI
OCS-T

FIGURE 26

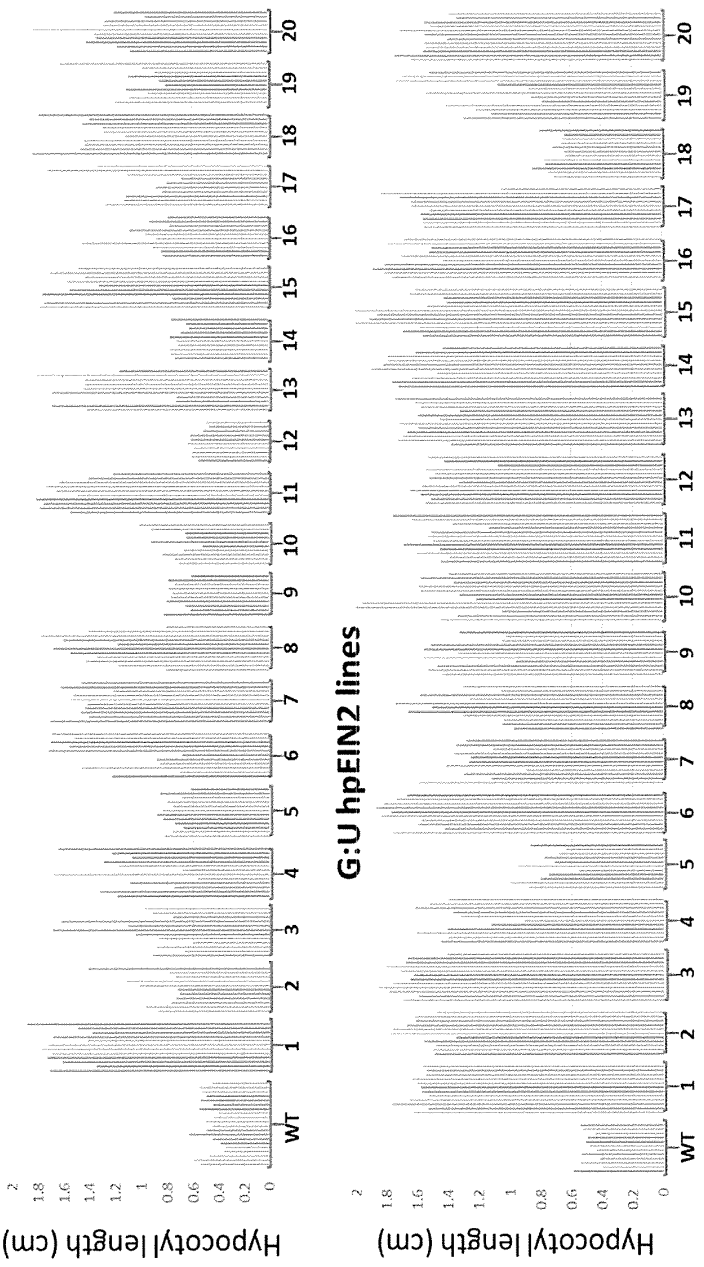

hpEIN2[wt] lines
G:U hpEIN2 lines
Hypocotyl length (cm)
Hypocotyl length (cm)
WT
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2

FIGURE 27

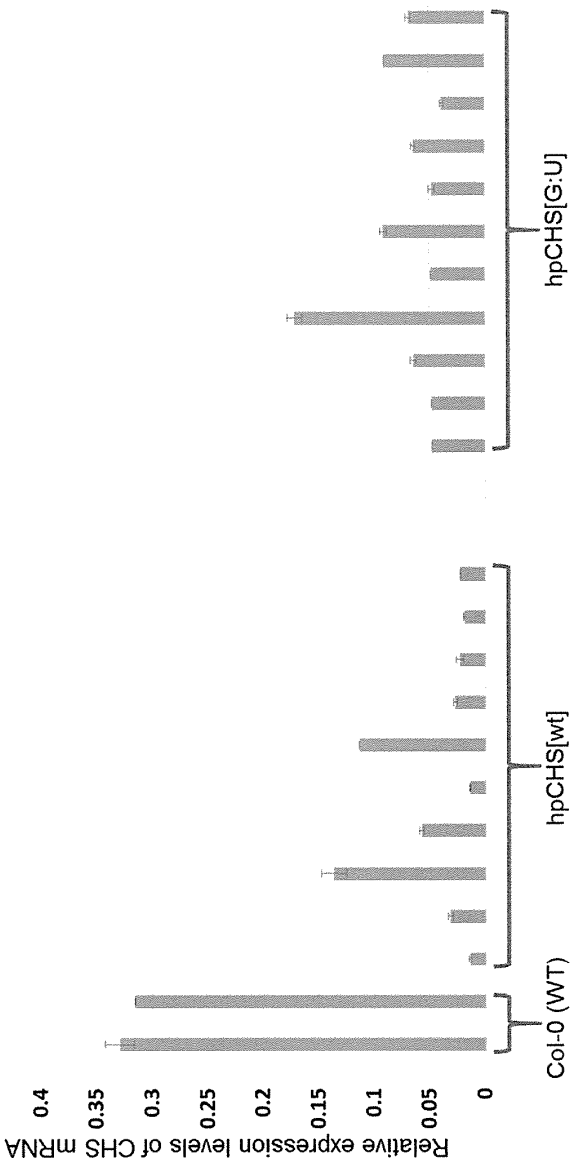

Relative expression levels of CHS mRNA
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Col-0 (WT)
hpCHS[wt]
hpCHS[G:U]

FIGURE 28

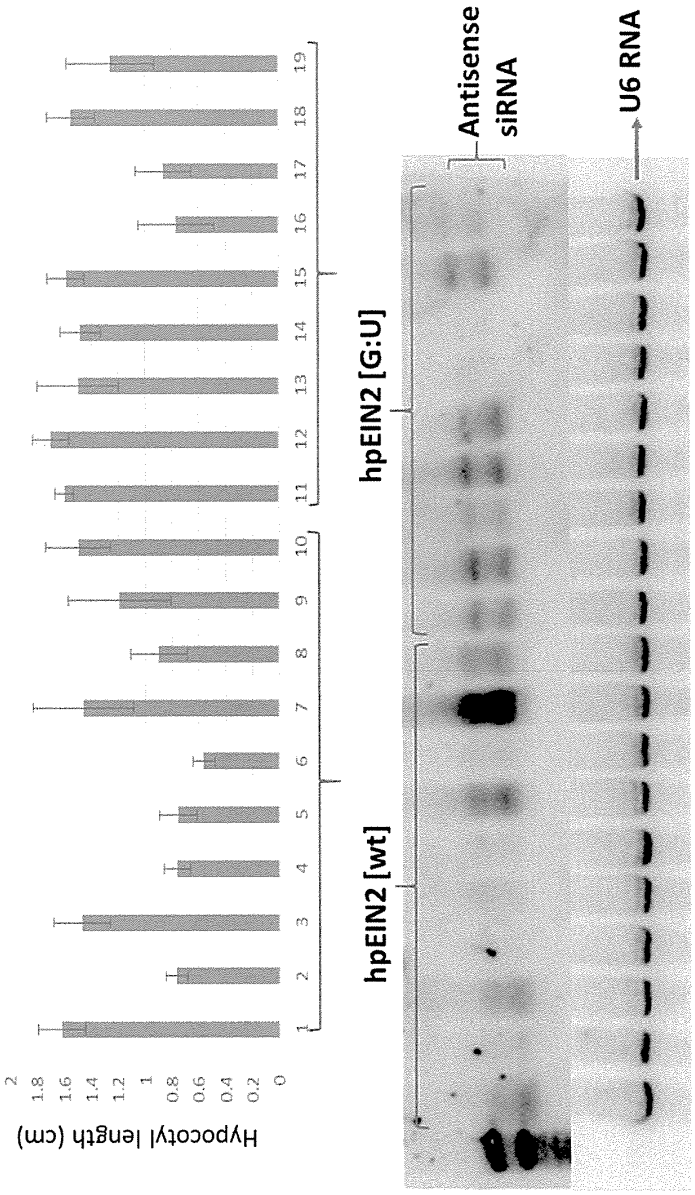

Hypocotyl length (cm)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
hpEIN2 [wt]
hpEIN2 [G:U]
Antisense siRNA
U6 RNA

FIGURE 29

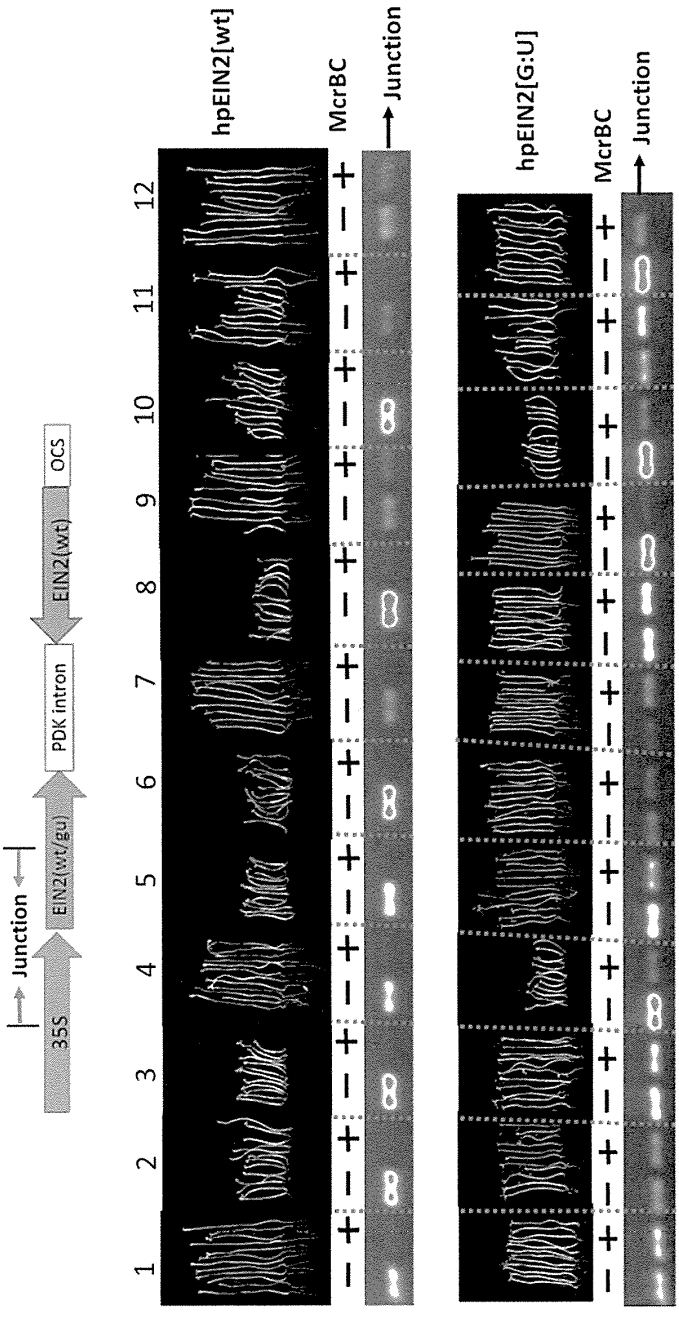

35S
Junction
EIN2(wt/gu)
PDK intron
EIN2(wt)
OCS
hpEIN2[wt]
McrBC
Junction
hpEIN2[G:U]
McrBC
Junction
1
2
3
4
5
6
7
8
9
10
11
12

FIGURE 30

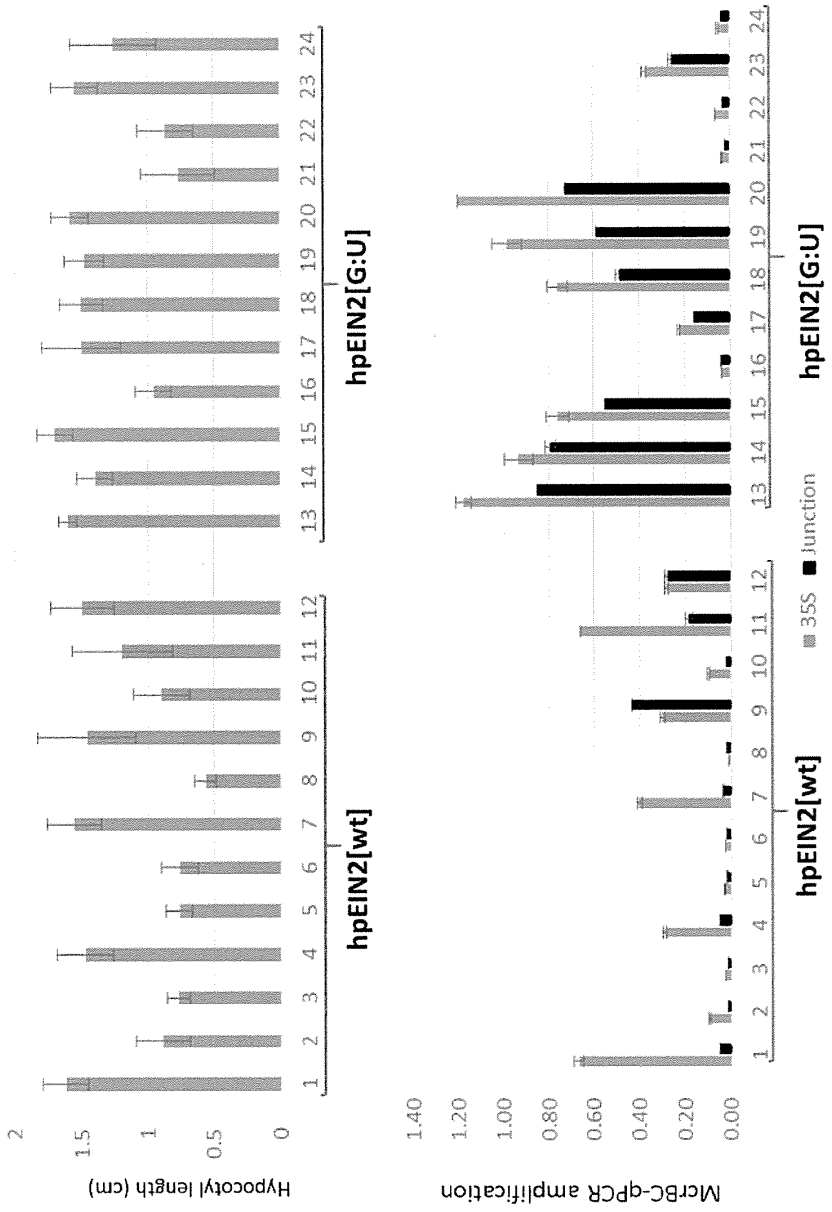

Hypocotyl length (cm)
0
0.5
1
1.5
2
1 2 3 4 5 6 7 8 9 10 11 12
hpEIN2[wt]
13 14 15 16 17 18 19 20 21 22 23 24
hpEIN2[G:U]
McrBC-qPCR amplification
0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
35S
Junction

FIGURE 31

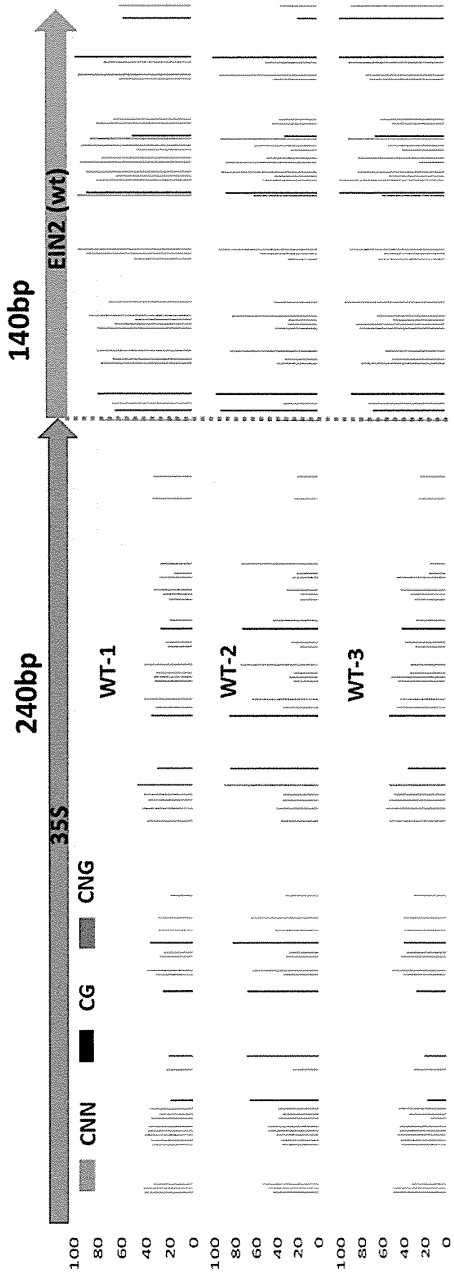


FIGURE 32

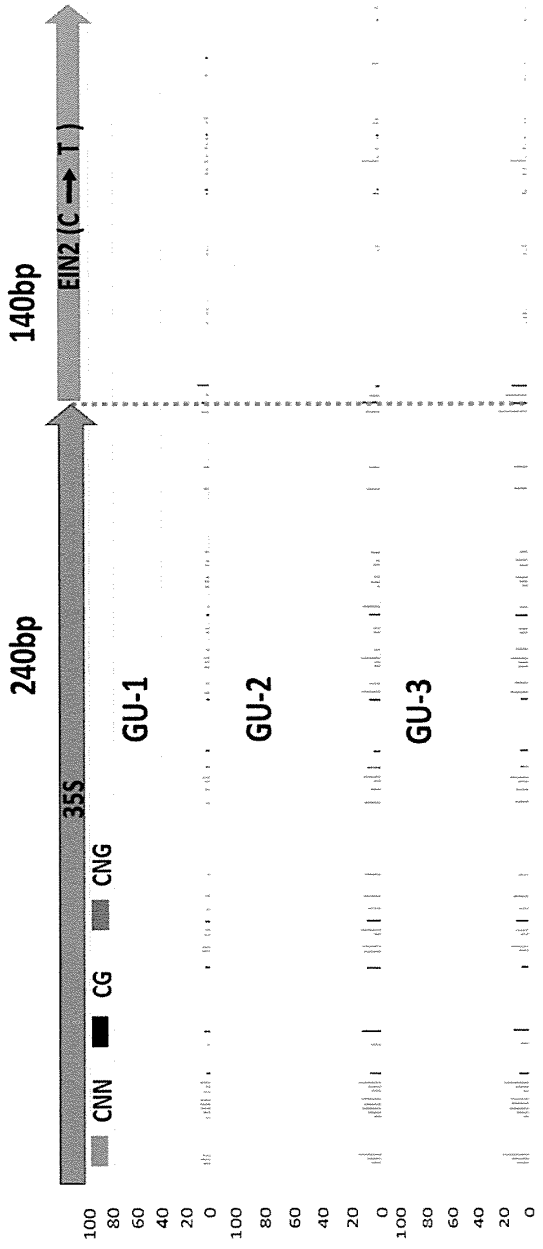


FIGURE 33

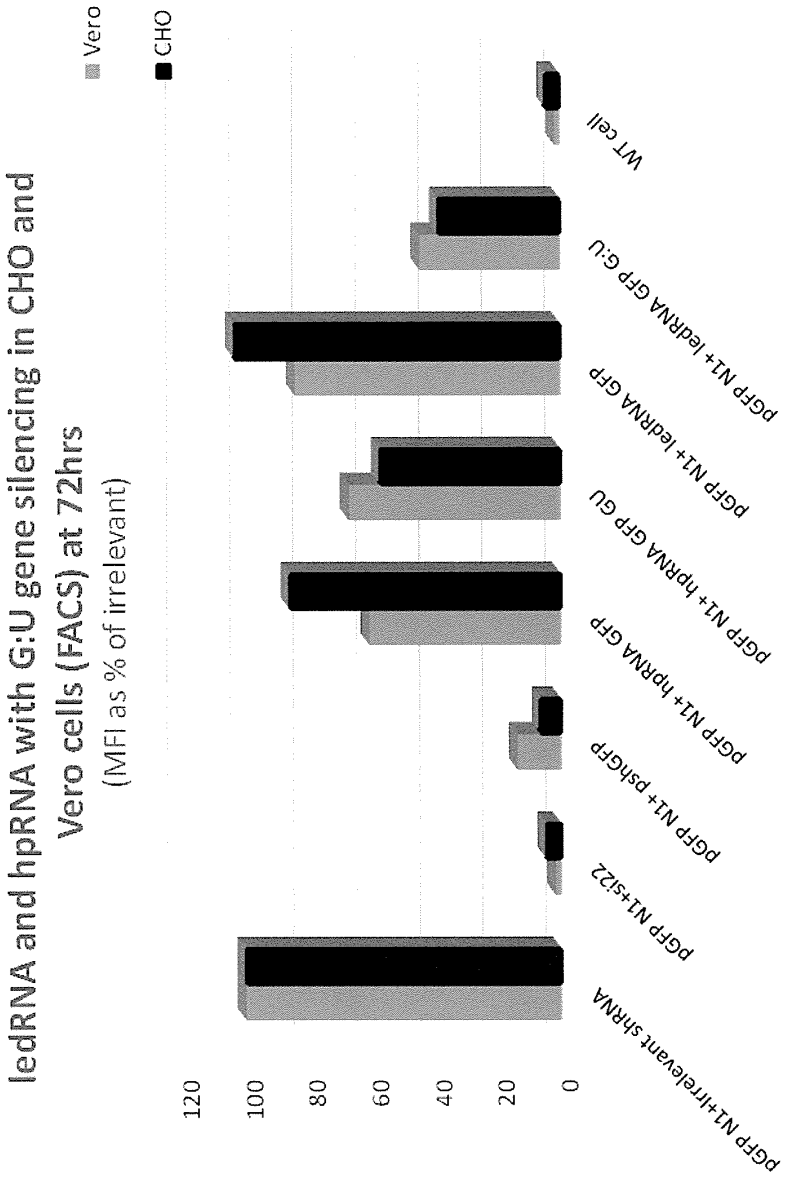

IedRNA and hpRNA with G:U gene silencing in CHO and Vero cells (FACS) at 72hrs
(MFI as % of irrelevant)
Vero
CHO
120
100
80
60
40
20
0
pGFP N1+Irrelevant shRNA
pGFP N1+si22
pGFP N1+ pshGFP
pGFP N1+ hpRNA GFP
pGFP N1+ hpRNA GFP GU
pGFP N1+ IedRNA GFP
pGFP N1+ IedRNA GFP G:U
WT cell

FIGURE 34

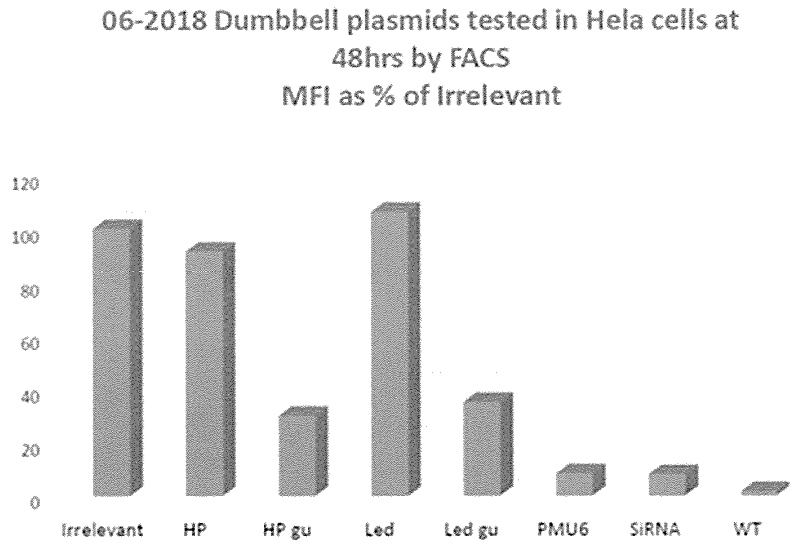

06-2018 Dumbbell plasmids tested in Hela cells at
48hrs by FACS
MFI as % of Irrelevant
120
100
80
60
40
20
0
Irrelevant
HP
HP gu
Led
Led gu
PMU6
SiRNA
WT

FIGURE 35

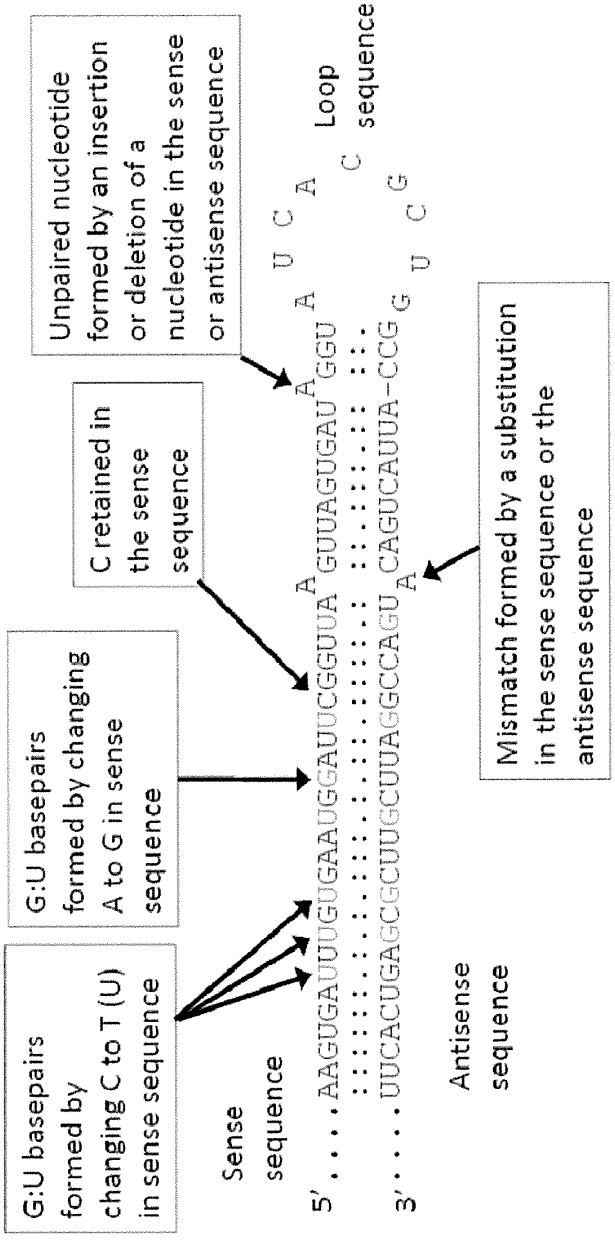
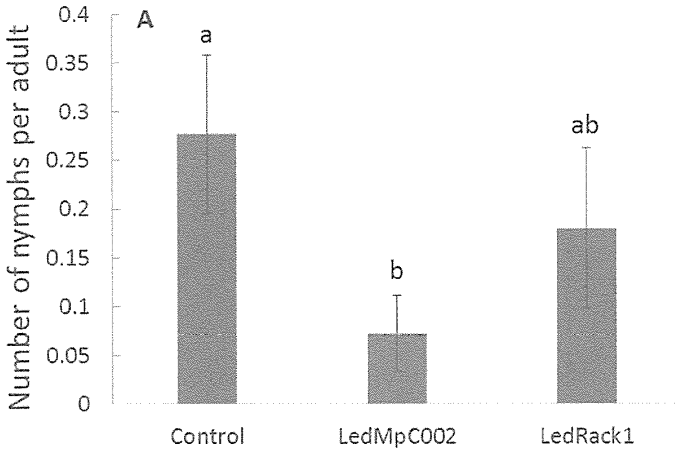

Unpaired nucleotide formed by an insertion or deletion of a nucleotide in the sense or antisense sequence
C retained in the sense sequence
G:U basepairs formed by changing A to G in sense sequence
G:U basepairs formed by changing C to T (U) in sense sequence
Mismatch formed by a substitution in the sense sequence or the antisense sequence
Loop sequence
Sense sequence
Antisense sequence
5'....AAGUGAUUUGUGAAUGGAUUCGGUUA GUUAGUGAU GGU
3'....UUCACUGAGCGCUUGCUUAGGCCAGU CAGUCAUUA-CCG

FIGURE 36

A


Number of nymphs per adult
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
A
a
b
ab
Control
LedMpC002
LedRack1

B

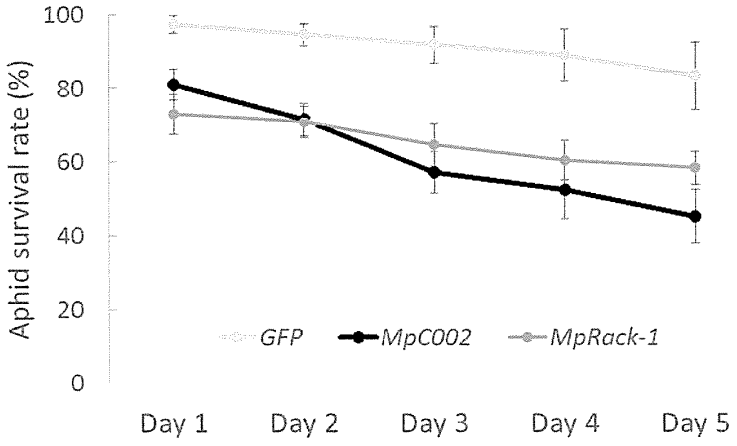

Aphid survival rate (%)
100
80
60
40
20
0
GFP
MpC002
MpRack-1
Day 1
Day 2
Day 3
Day 4
Day 5

FIGURE 37