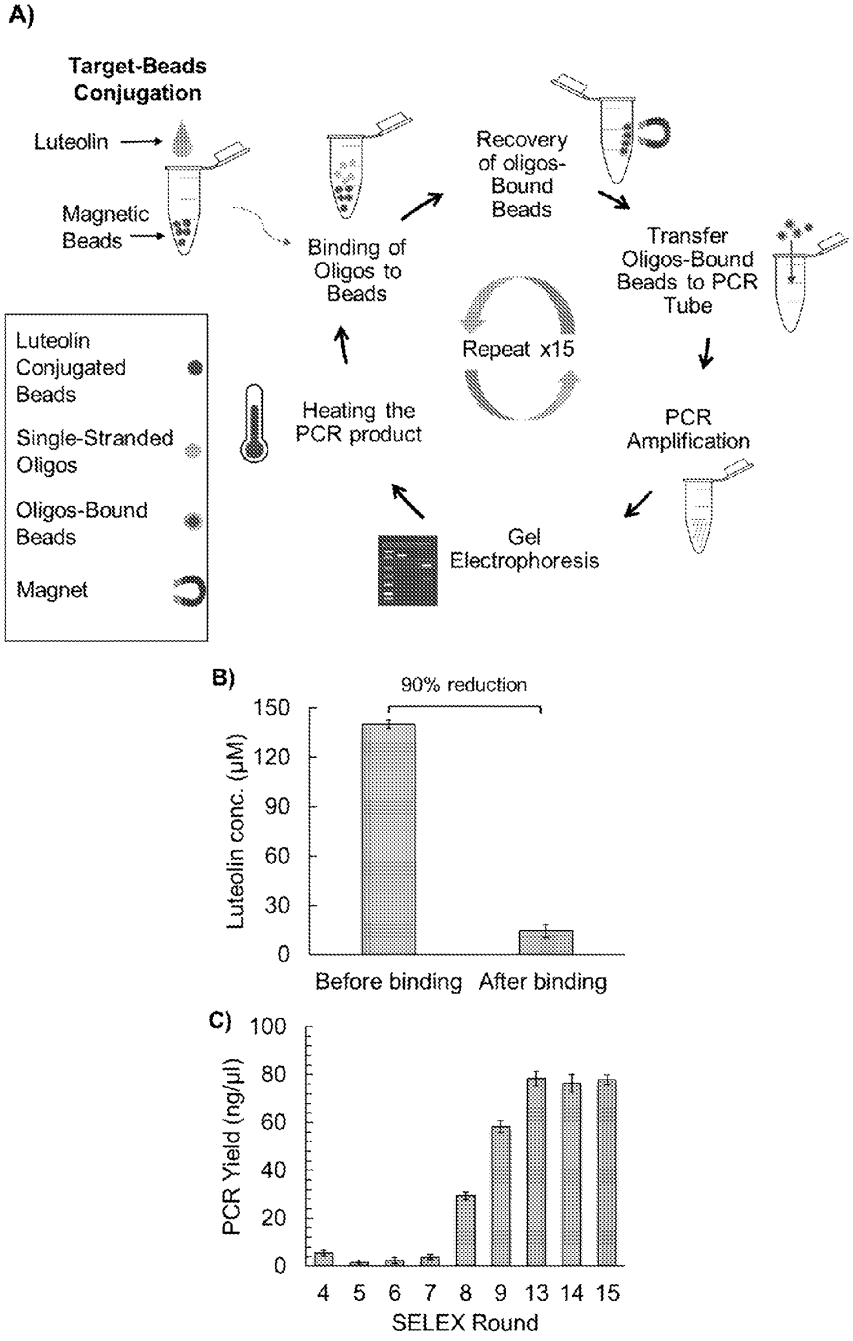

A)
Target-Beads Conjugation
Luteolin
Magnetic Beads
Binding of Oligos to Beads
Recovery of oligos-Bound Beads
Transfer Oligos-Bound Beads to PCR Tube
PCR Amplification
Gel Electrophoresis
Heating the PCR product
Repeat x15
Luteolin Conjugated Beads
Single-Stranded Oligos
Oligos-Bound Beads
Magnet
B)
90% reduction
Luteolin conc. (μM)
150
120
90
60
30
0
Before binding
After binding
C)
PCR Yield (ng/μl)
100
80
60
40
20
0
4 5 6 7 8 9 13 14 15
SELEX Round

**Figure 1**

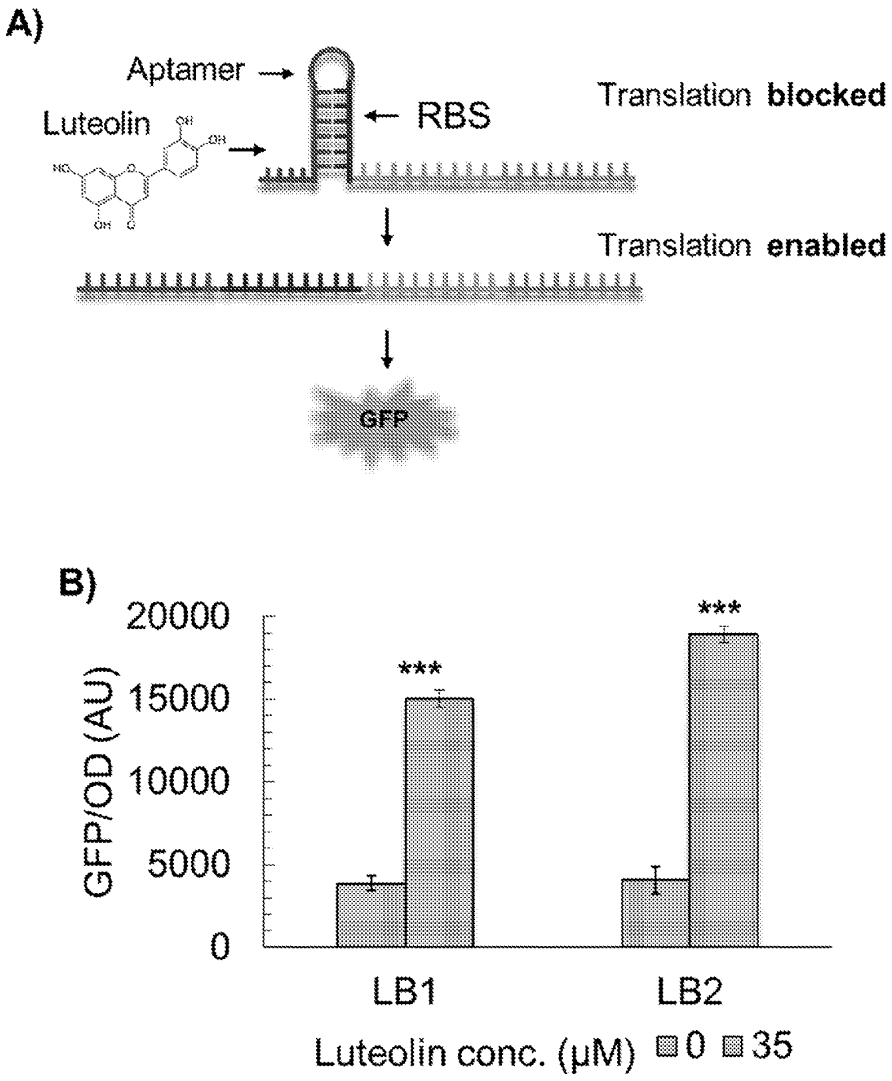

A)
Aptamer
Luteolin
RBS
Translation blocked
Translation enabled
GFP
B)
GFP/OD (AU)
20000
15000
10000
5000
0
***
***
LB1
LB2
Luteolin conc. (μM) 0 35

**Figure 2**

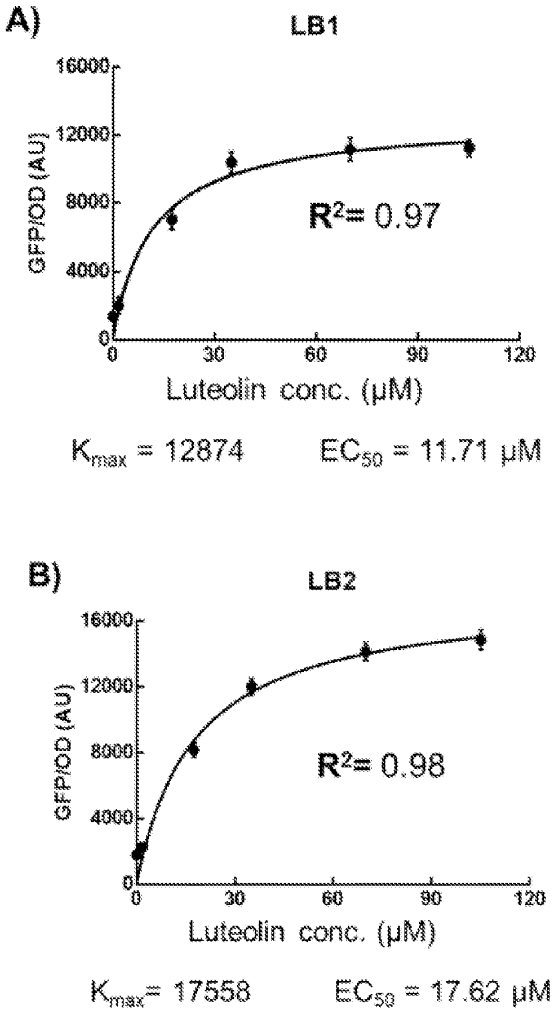

A)
LB1
16000
12000
8000
4000
0
GFP/OD (AU)
R²= 0.97
0
30
60
90
120
Luteolin conc. (μM)
K_max = 12874
EC_50 = 11.71 μM
B)
LB2
16000
12000
8000
4000
0
GFP/OD (AU)
R²= 0.98
0
30
60
90
120
Luteolin conc. (μM)
K_max= 17558
EC_50 = 17.62 μM

**Figure 3**

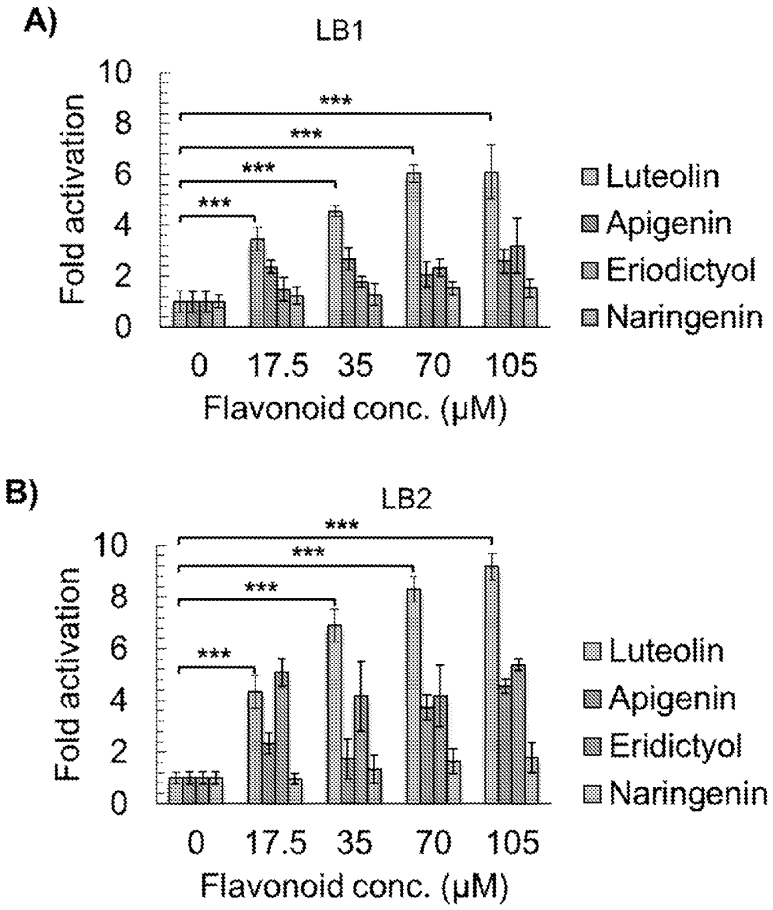

A)
LB1
Fold activation
10
8
6
4
2
0
***
***
***
***
0
17.5
35
70
105
Flavonoid conc. (μM)
Luteolin
Apigenin
Eriodictyol
Naringenin
B)
LB2
Fold activation
10
8
6
4
2
0
***
***
***
***
0
17.5
35
70
105
Flavonoid conc. (μM)
Luteolin
Apigenin
Eridictyol
Naringenin

**Figure 4**

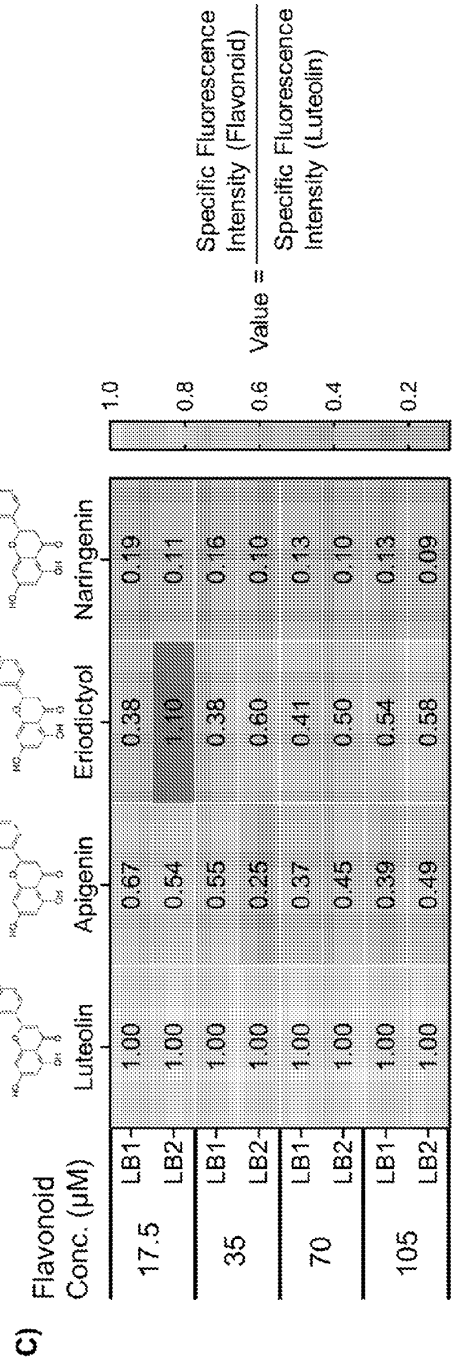



**Figure 4 (continued)**

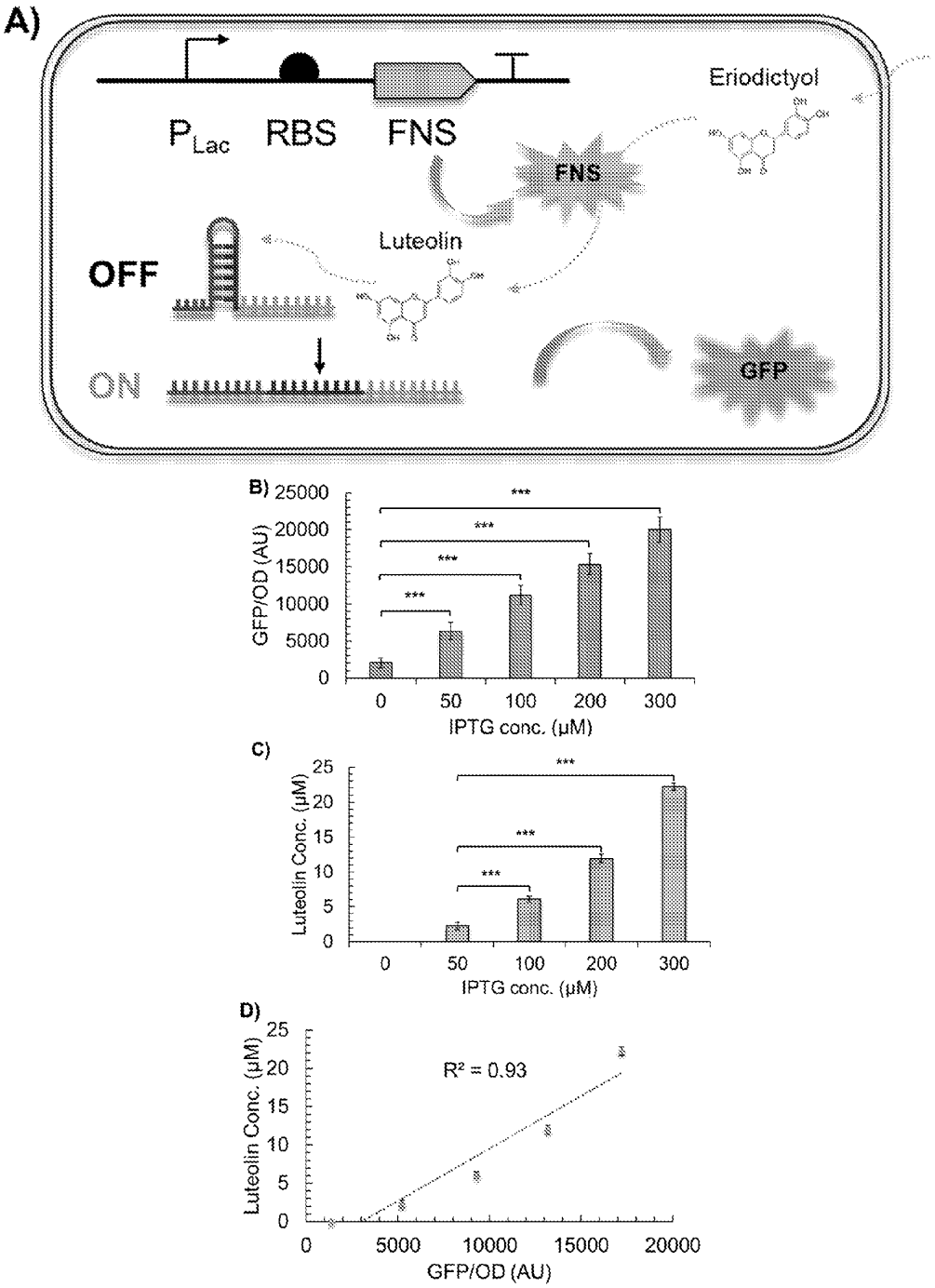

A)
P Lac
RBS
FNS
Eriodictyol
FNS
Luteolin
OFF
ON
GFP
B)
GFP/OD (AU)
25000
20000
15000
10000
5000
0
0
50
100
200
300
IPTG conc. (μM)
***
C)
Luteolin Conc. (μM)
25
20
15
10
5
0
IPTG conc. (μM)
D)
Luteolin Conc. (μM)
R² = 0.93
5000
10000
15000
20000
GFP/OD (AU)

**Figure 5**

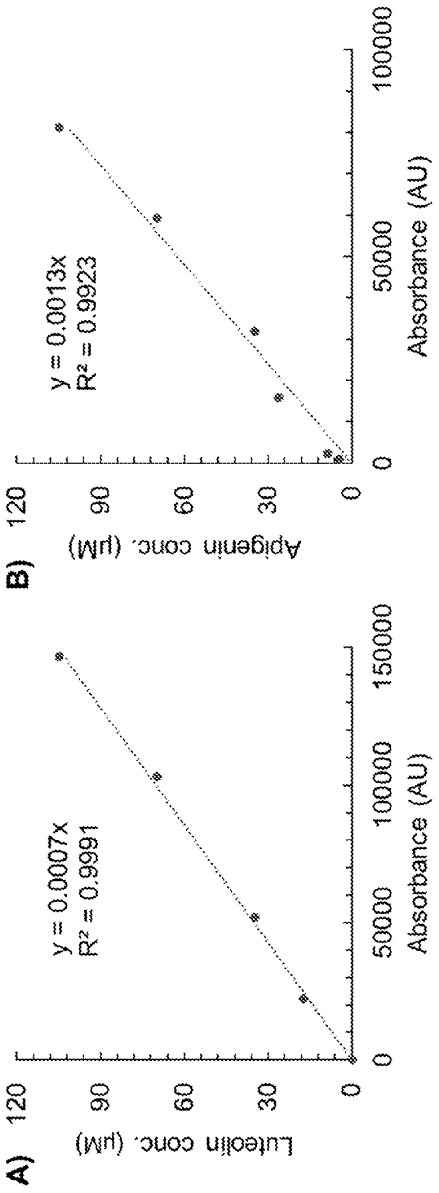

A)
Luteolin conc. (μM)
120
90
60
30
0
y = 0.0007x
R² = 0.9991
0
50000
100000
150000
Absorbance (AU)
B)
Apigenin conc. (μM)
120
90
60
30
0
y = 0.0013x
R² = 0.9923
0
50000
100000
Absorbance (AU)

**Figure 6**

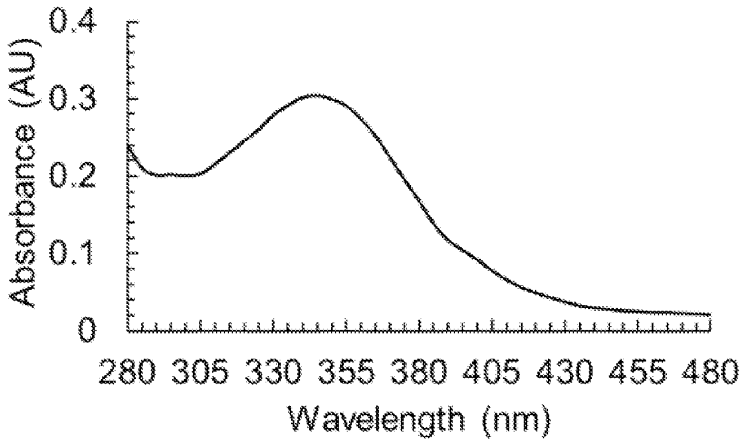

Absorbance (AU)
0.4
0.3
0.2
0.1
0
280 305 330 355 380 405 430 455 480
Wavelength (nm)

**Figure 7**

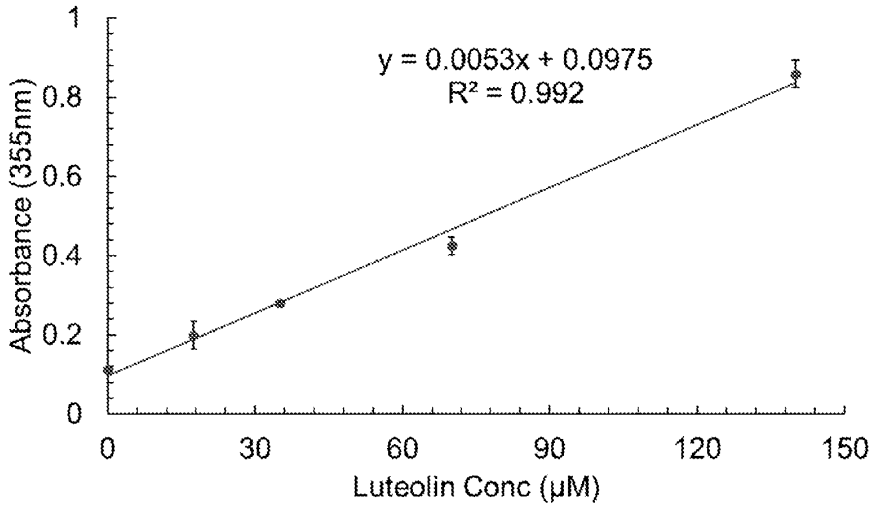

y = 0.0053x + 0.0975
R² = 0.992
Absorbance (355nm)
1
0.8
0.6
0.4
0.2
0
0
30
60
90
120
150
Luteolin Conc (µM)

**Figure 8**

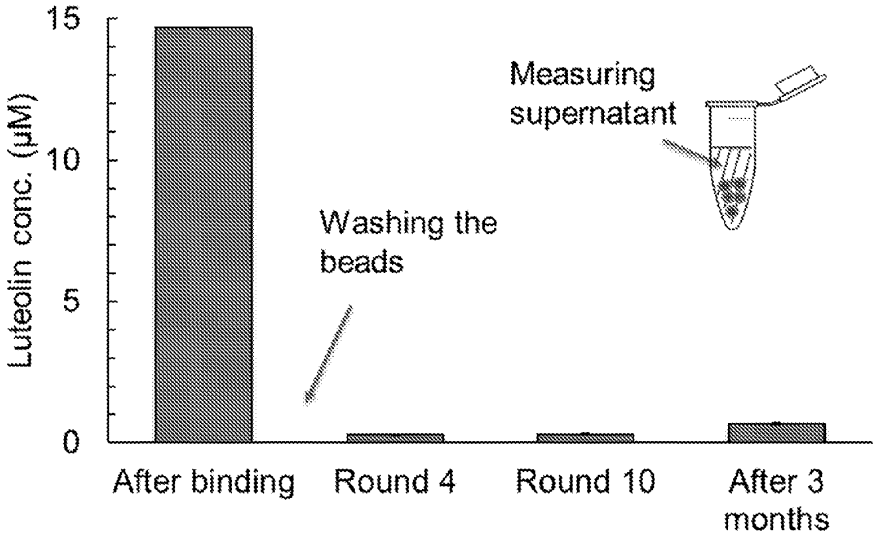

15
10
5
0
Luteolin conc. (μM)
Measuring supernatant
Washing the beads
After binding
Round 4
Round 10
After 3 months

**Figure 9**

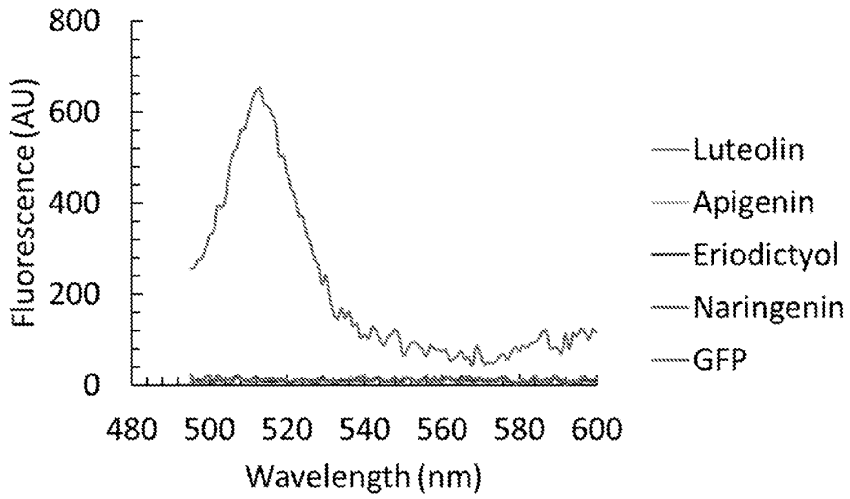

800
600
400
200
0
Fluorescence (AU)
480 500 520 540 560 580 600
Wavelength (nm)
Luteolin
Apigenin
Eriodictyol
Naringenin
GFP

**Figure 10**

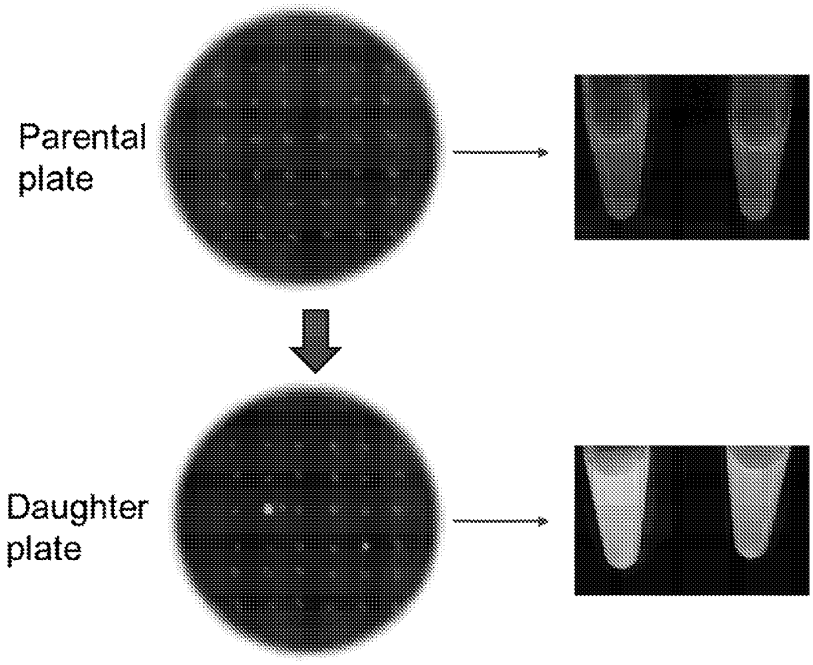



**Figure 11**

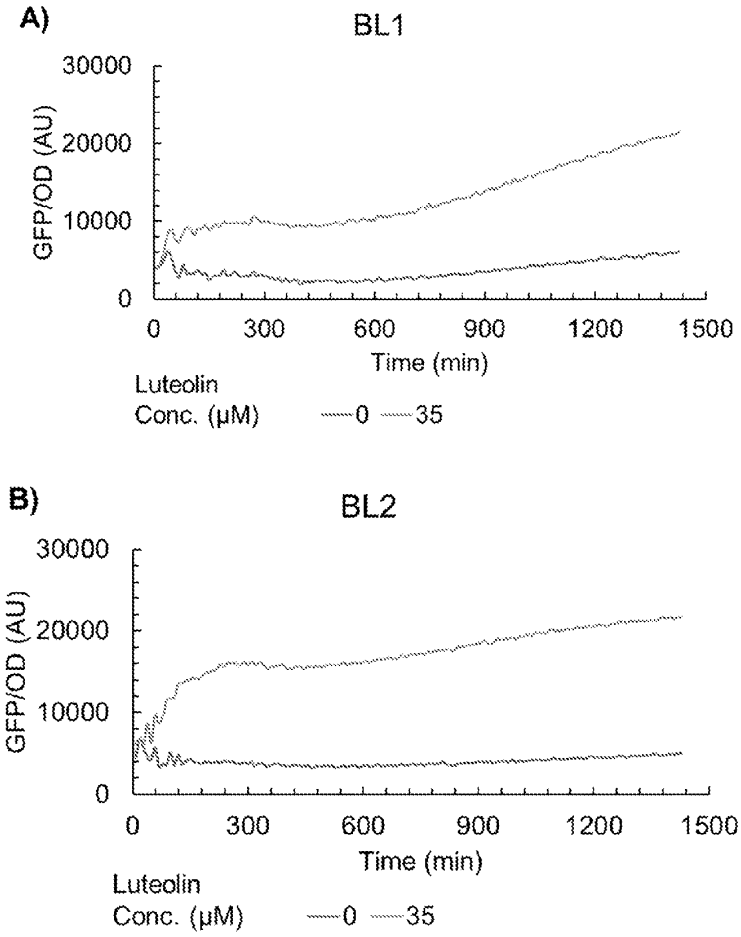

A)
BL1
30000
20000
10000
0
GFP/OD (AU)
0
300
600
900
1200
1500
Time (min)
Luteolin
Conc. (μM)
0
35
B)
BL2
30000
20000
10000
0
GFP/OD (AU)
0
300
600
900
1200
1500
Time (min)
Luteolin
Conc. (μM)
0
35

**Figure 12**

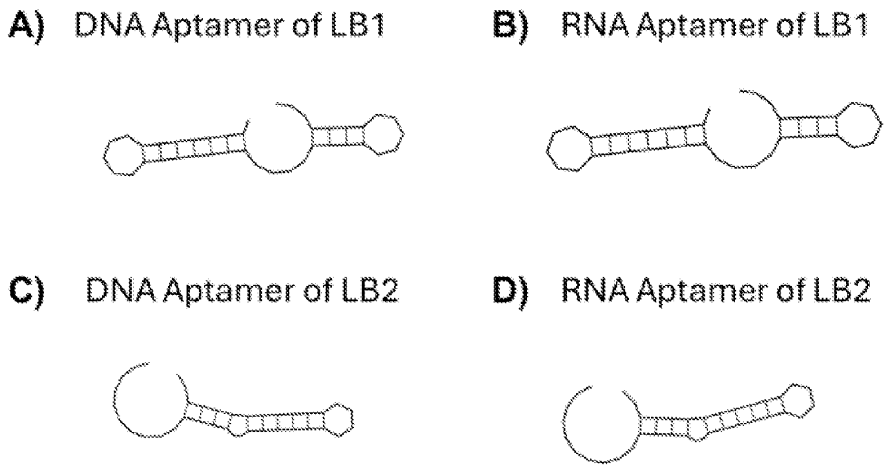

A) DNA Aptamer of LB1
B) RNA Aptamer of LB1
C) DNA Aptamer of LB2
D) RNA Aptamer of LB2

**Figure 13**

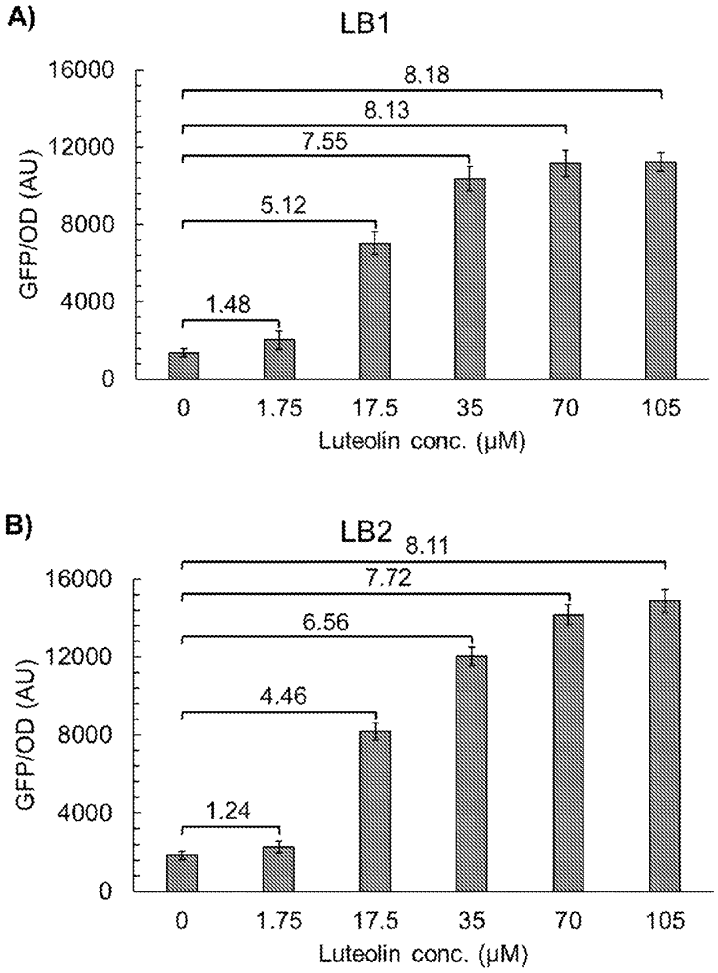

A)
LB1
16000
12000
8000
4000
0
GFP/OD (AU)
8.18
8.13
7.55
5.12
1.48
0
1.75
17.5
35
70
105
Luteolin conc. (μM)
B)
LB2
8.11
7.72
6.56
4.46
1.24
16000
12000
8000
4000
0
GFP/OD (AU)
0
1.75
17.5
35
70
105
Luteolin conc. (μM)

**Figure 14**

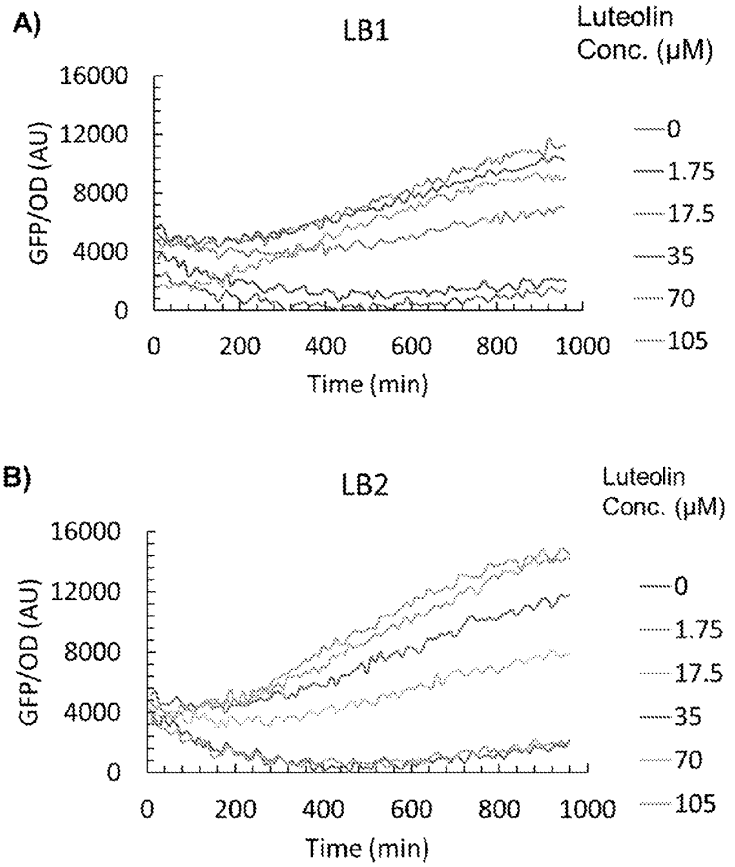

A)
LB1
Luteolin
Conc. (μM)
16000
12000
8000
4000
0
GFP/OD (AU)
0
200
400
600
800
1000
Time (min)
0
1.75
17.5
35
70
105
B)
LB2
Luteolin
Conc. (μM)
16000
12000
8000
4000
0
GFP/OD (AU)
0
200
400
600
800
1000
Time (min)
0
1.75
17.5
35
70
105

**Figure 15**

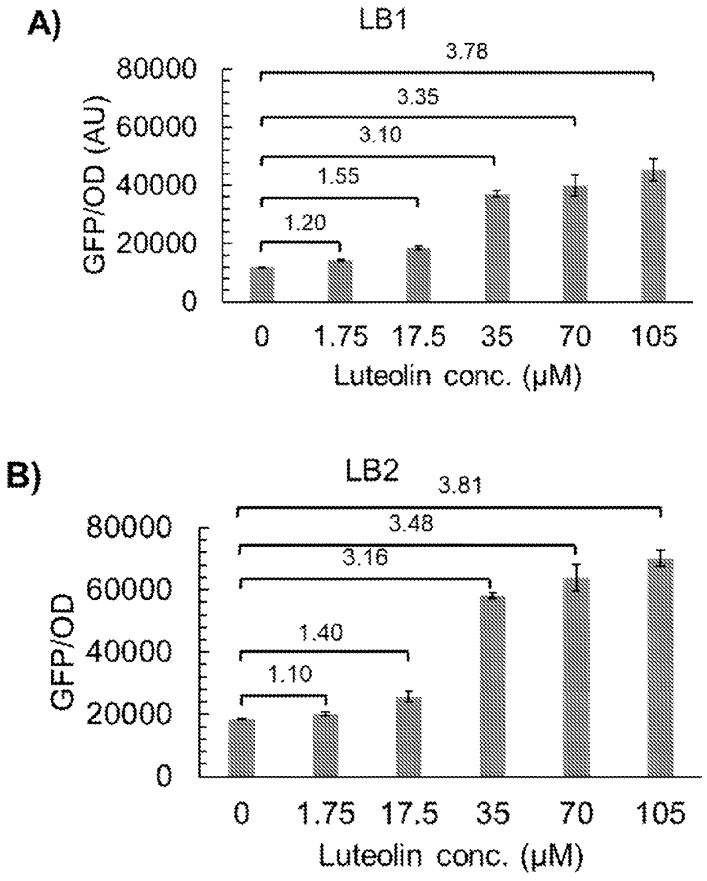

A)
LB1
GFP/OD (AU)
80000
60000
40000
20000
0
3.78
3.35
3.10
1.55
1.20
0
1.75
17.5
35
70
105
Luteolin conc. (μM)
B)
LB2
GFP/OD
80000
60000
40000
20000
0
3.81
3.48
3.16
1.40
1.10
0
1.75
17.5
35
70
105
Luteolin conc. (μM)

**Figure 16**

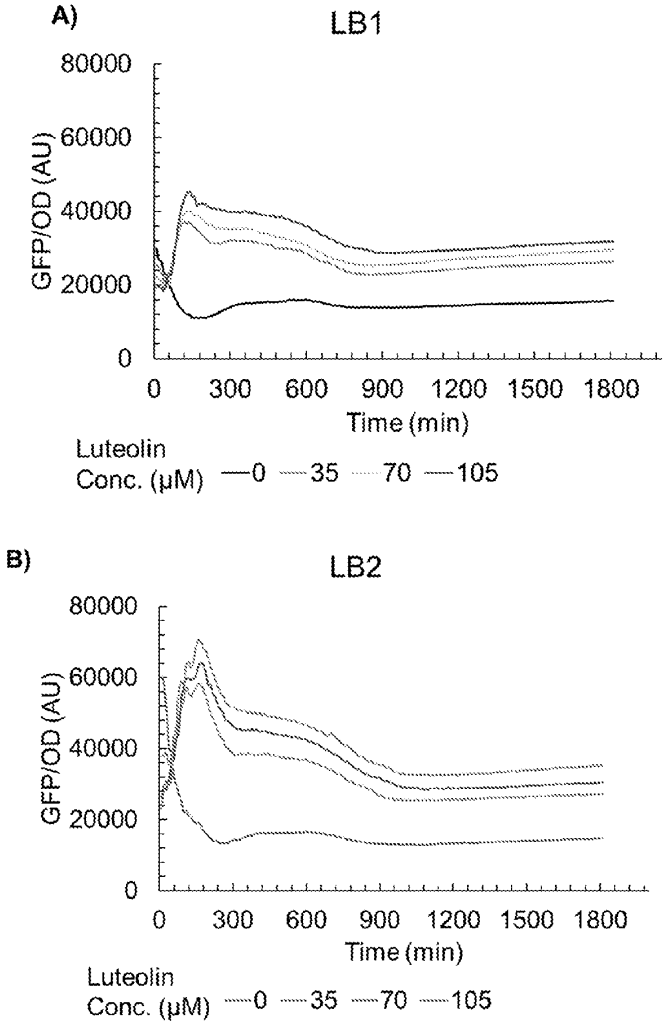

A)
LB1
80000
60000
40000
20000
0
GFP/OD (AU)
0
300
600
900
1200
1500
1800
Time (min)
Luteolin
Conc. (μM)
0
35
70
105
B)
LB2
80000
60000
40000
20000
0
GFP/OD (AU)
0
300
600
900
1200
1500
1800
Time (min)
Luteolin
Conc. (μM)
0
35
70
105

**Figure 17**

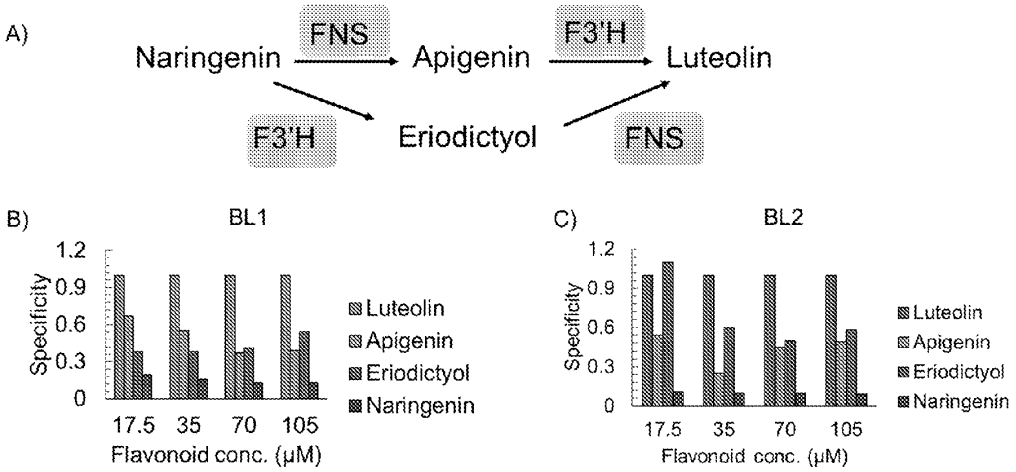
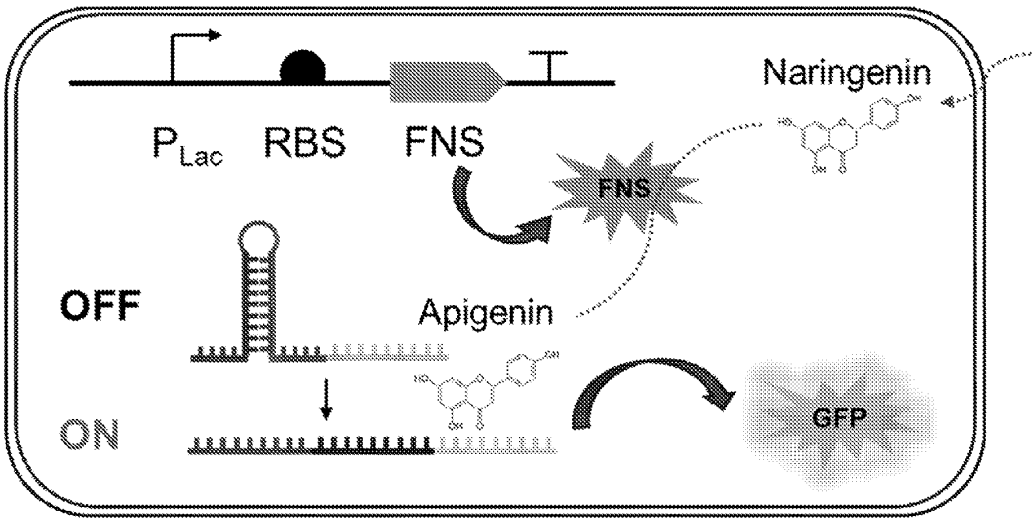
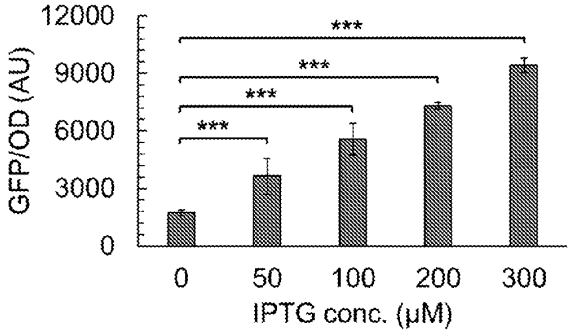

A)
Naringenin
FNS
Apigenin
F3'H
Luteolin
F3'H
Eriodictyol
FNS
B)
BL1
Specificity
1.2
0.9
0.6
0.3
0
17.5
35
70
105
Flavonoid conc. (μM)
Luteolin
Apigenin
Eriodictyol
Naringenin
C)
BL2
Specificity
1.2
0.9
0.6
0.3
0
17.5
35
70
105
Flavonoid conc. (μM)
Luteolin
Apigenin
Eriodictyol
Naringenin

**Figure 18**

A.


Naringenin
$P_{Lac}$ RBS FNS
FNS
OFF
Apigenin
ON
GFP

B.


12000
9000
6000
3000
0
GFP/OD (AU)
***
***
***
***
0 50 100 200 300
IPTG conc. (μM)

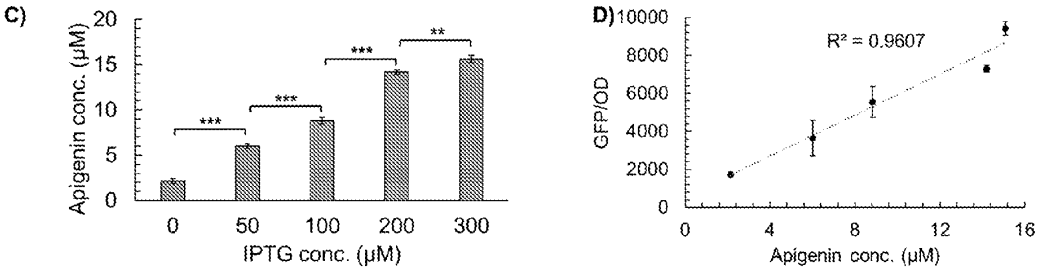

C)
20
15
10
5
0
Apigenin conc. (μM)
***
***
***
**
0 50 100 200 300
IPTG conc. (μM)
D)
10000
8000
6000
4000
2000
0
GFP/OD
R² = 0.9607
0 4 8 12 16
Apigenin conc. (μM)

**Figure 19**

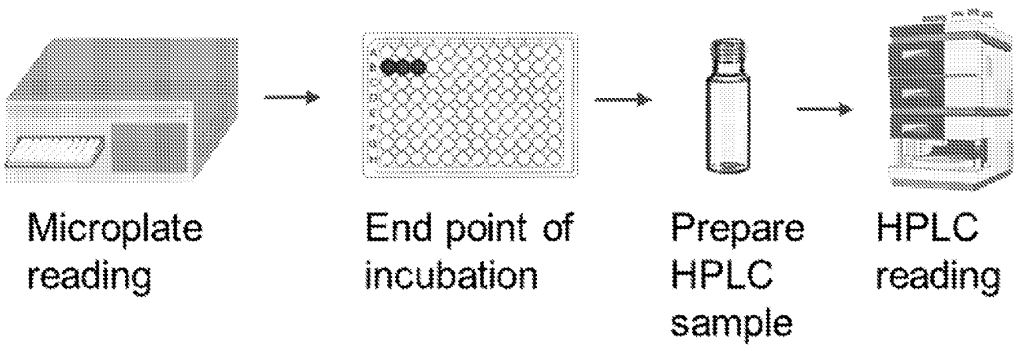
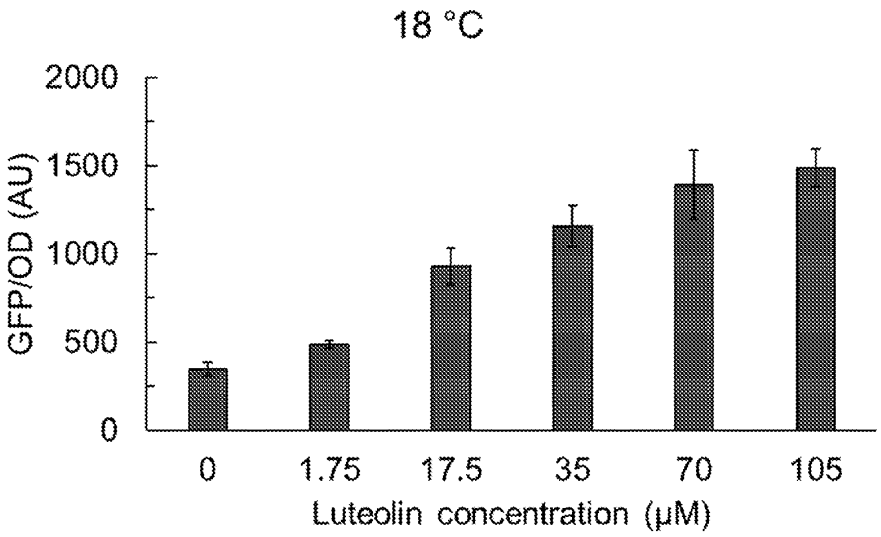

Microplate reading
End point of incubation
Prepare HPLC sample
HPLC reading

**Figure 20**

A


18 °C
GFP/OD (AU)
2000
1500
1000
500
0
0
1.75
17.5
35
70
105
Luteolin concentration (μM)

B

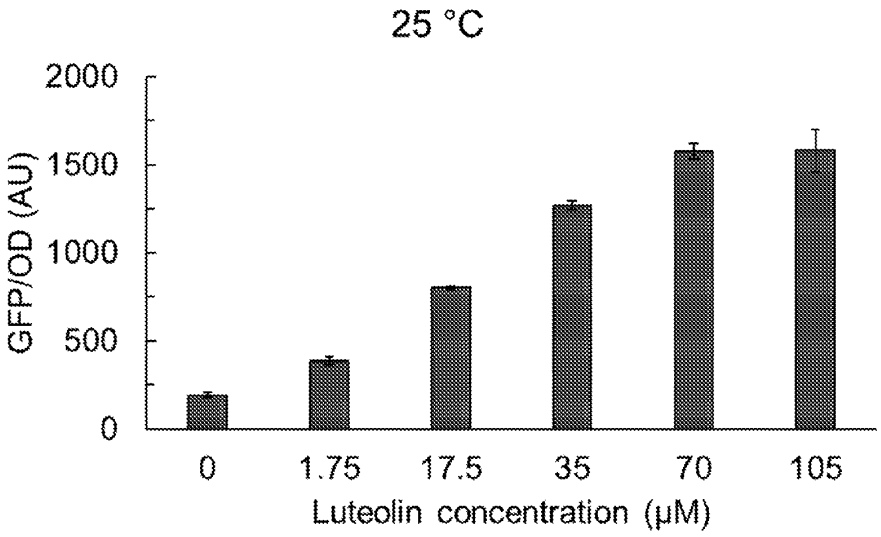
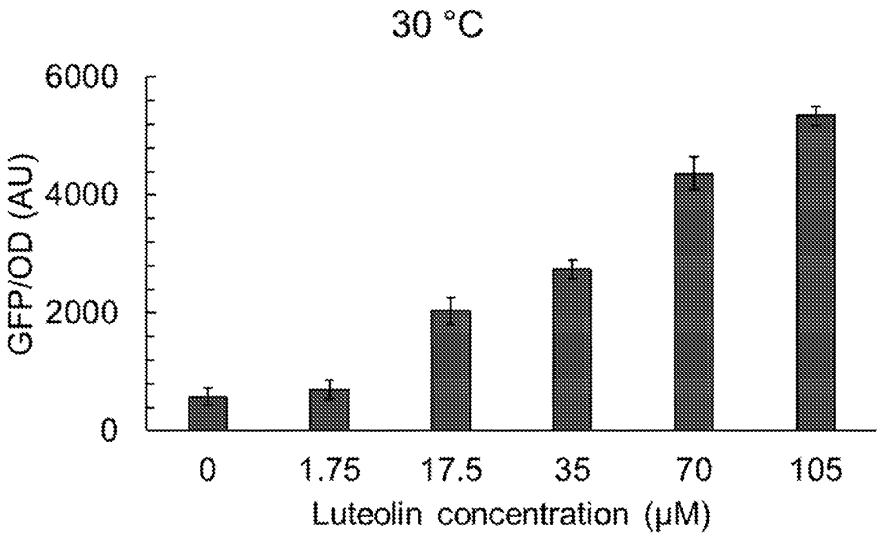

25 °C
GFP/OD (AU)
2000
1500
1000
500
0
0
1.75
17.5
35
70
105
Luteolin concentration (μM)

Figure 21

C




D

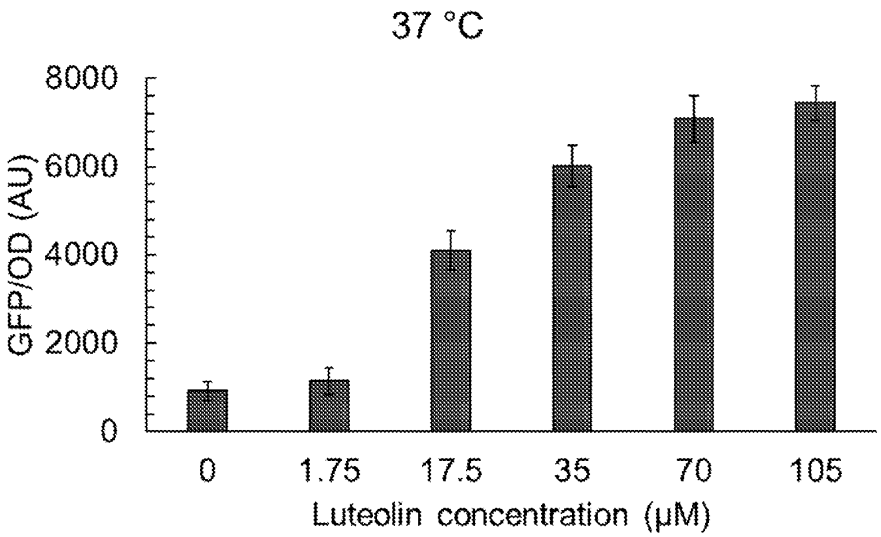



**Figure 21 (continued)**

E

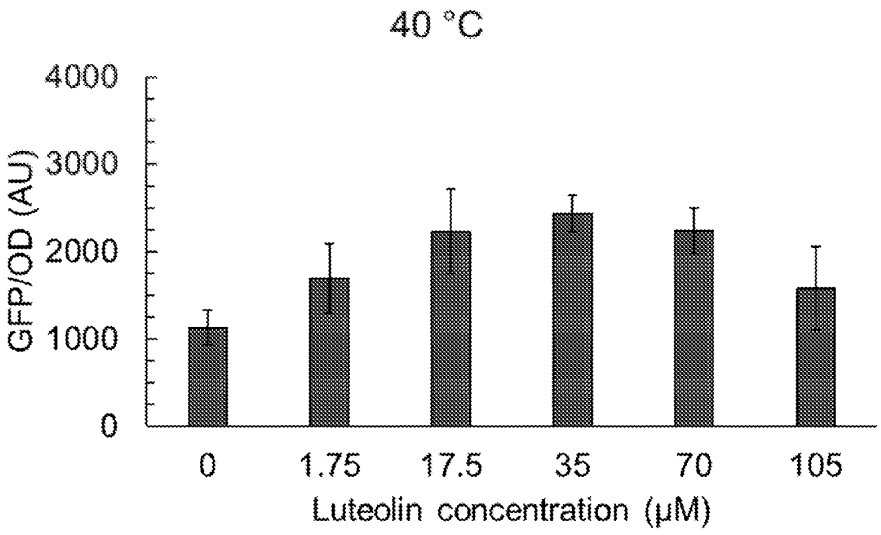



**Figure 21 (continued)**