Figure 1

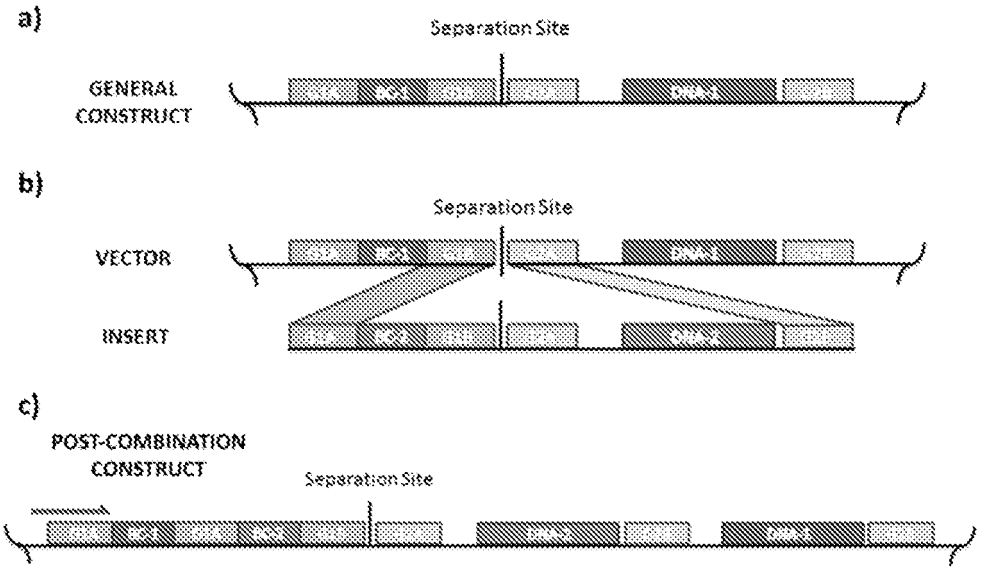

a)
Separation Site
GENERAL CONSTRUCT
b)
Separation Site
VECTOR
INSERT
c)
POST-COMBINATION CONSTRUCT
Separation Site

Figure 2

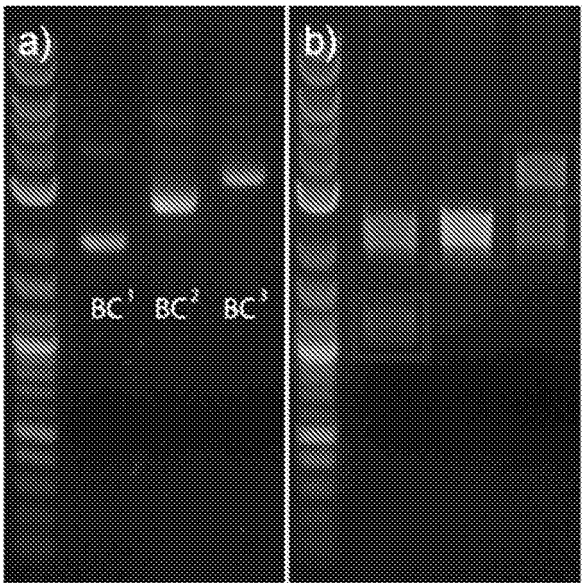


Figure 3

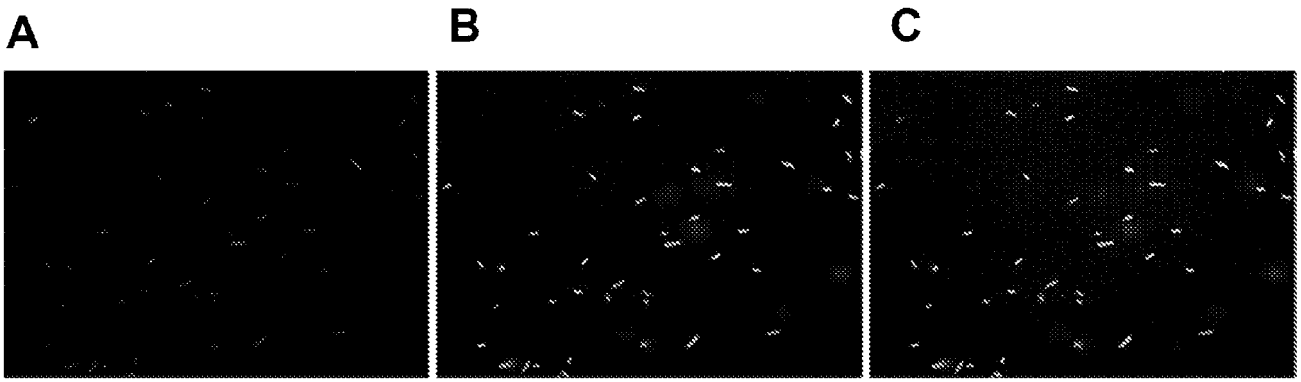


Figure 4

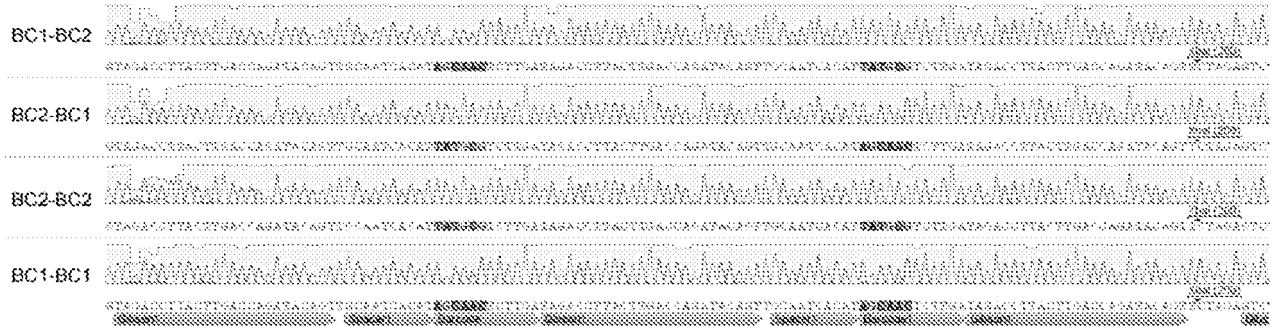


Figure 5

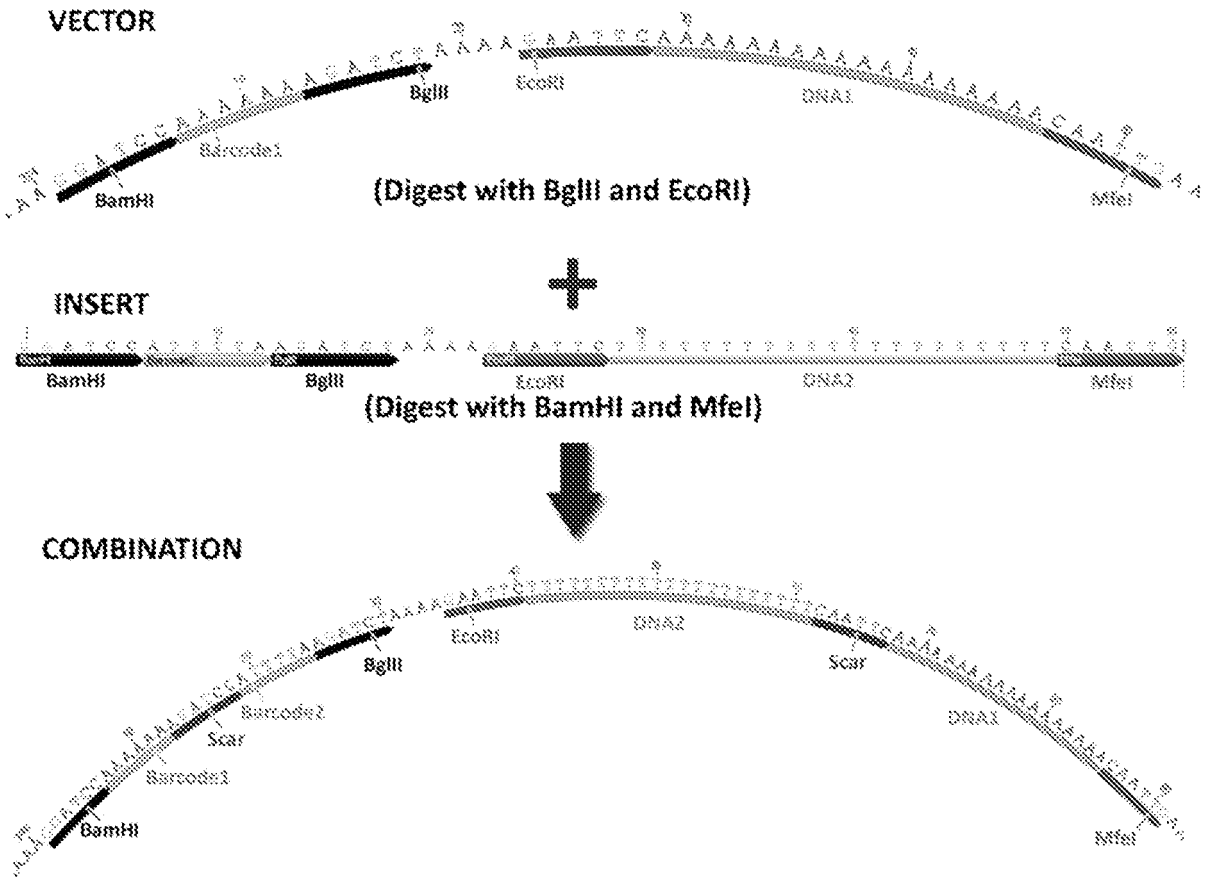

VECTOR
BglII
EcoRI
DNA1
Barcode1
BamHI
(Digest with BglII and EcoRI)
MfeI
INSERT
BamHI
BglII
EcoRI
DNA2
MfeI
(Digest with BamHI and MfeI)
COMBINATION
EcoRI
DNA2
BglII
Scar
Barcode2
Scar
DNA1
Barcode1
BamHI
MfeI

Figure 6

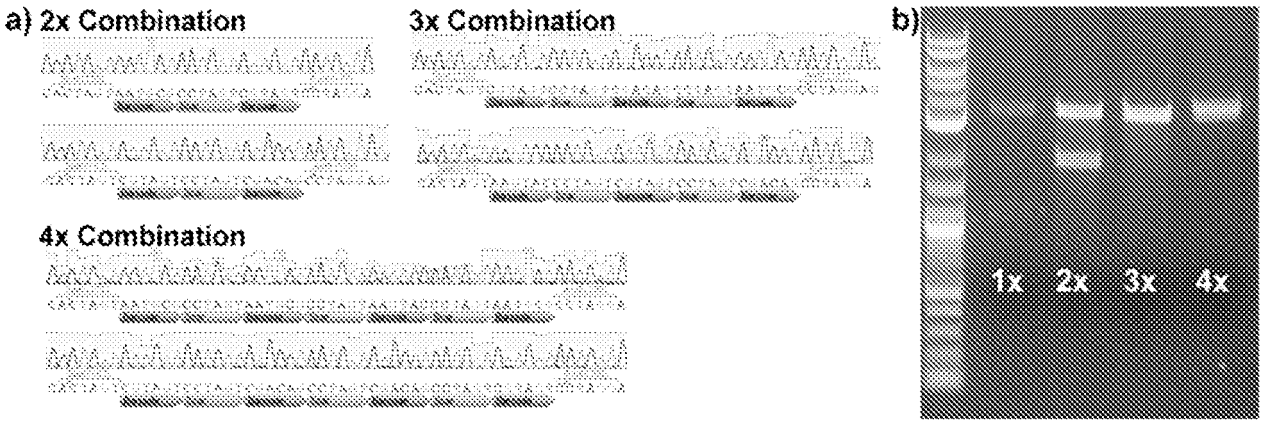


Figure 7

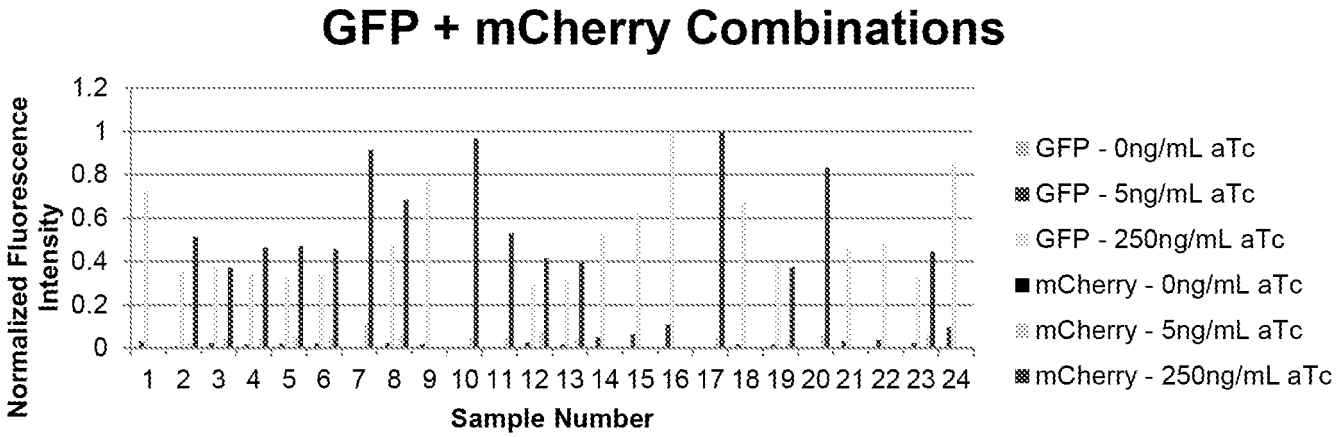

GFP + mCherry Combinations
Normalized Fluorescence Intensity
1.2
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Sample Number
GFP - 0ng/mL aTc
GFP - 5ng/mL aTc
GFP - 250ng/mL aTc
mCherry - 0ng/mL aTc
mCherry - 5ng/mL aTc
mCherry - 250ng/mL aTc

Figure 8

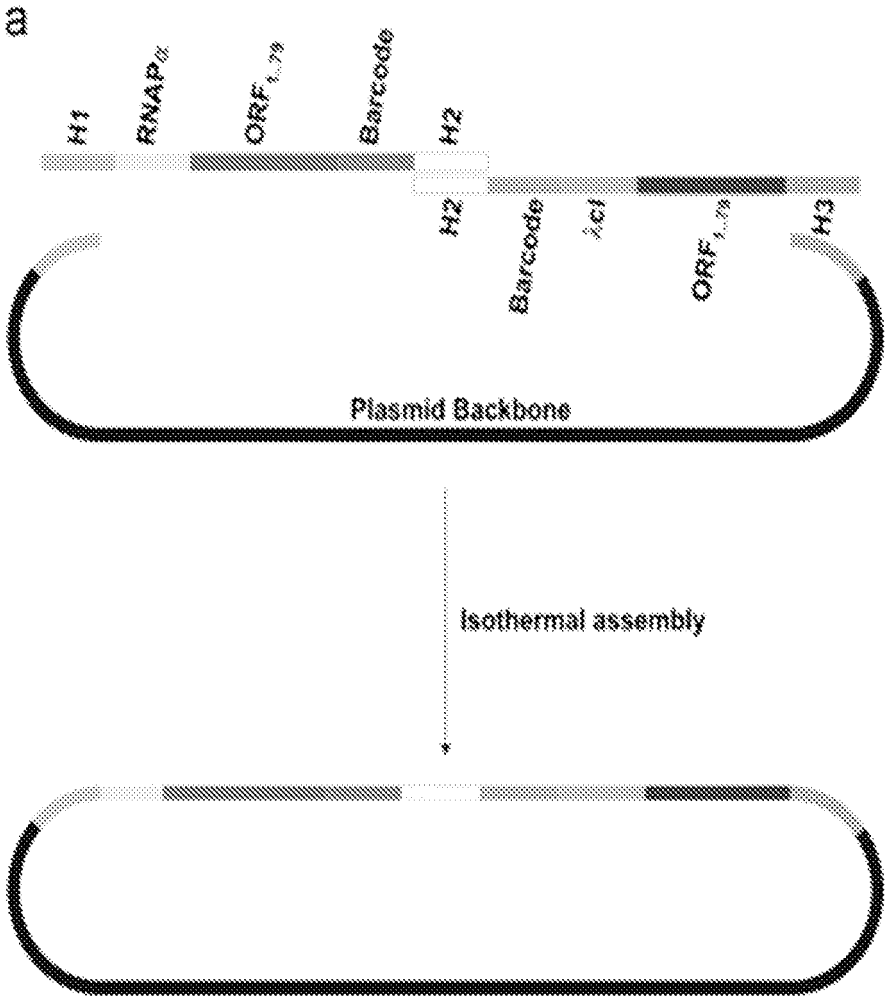
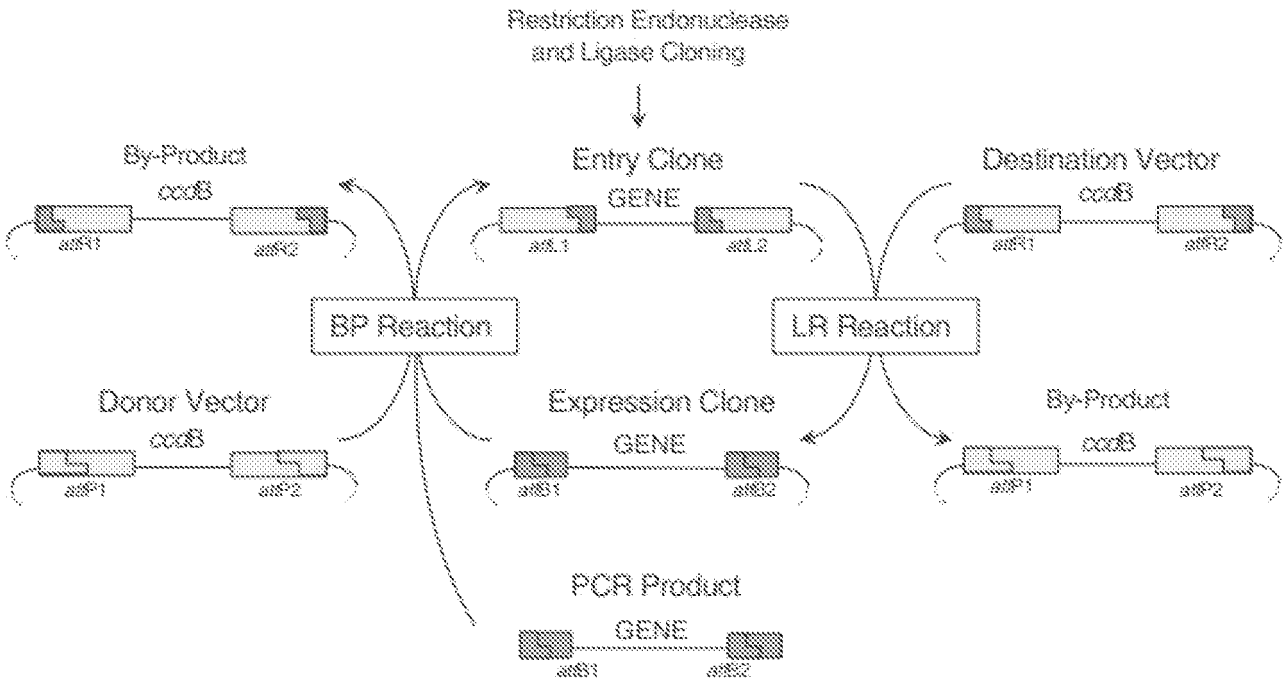

Ω
H1
RNAP$_\Omega$
ORF$_{1-79}$
Barcode
H2
H2
Barcode
λcI
ORF$_{1-79}$
H3
Plasmid Backbone
Isothermal assembly

Figure 9



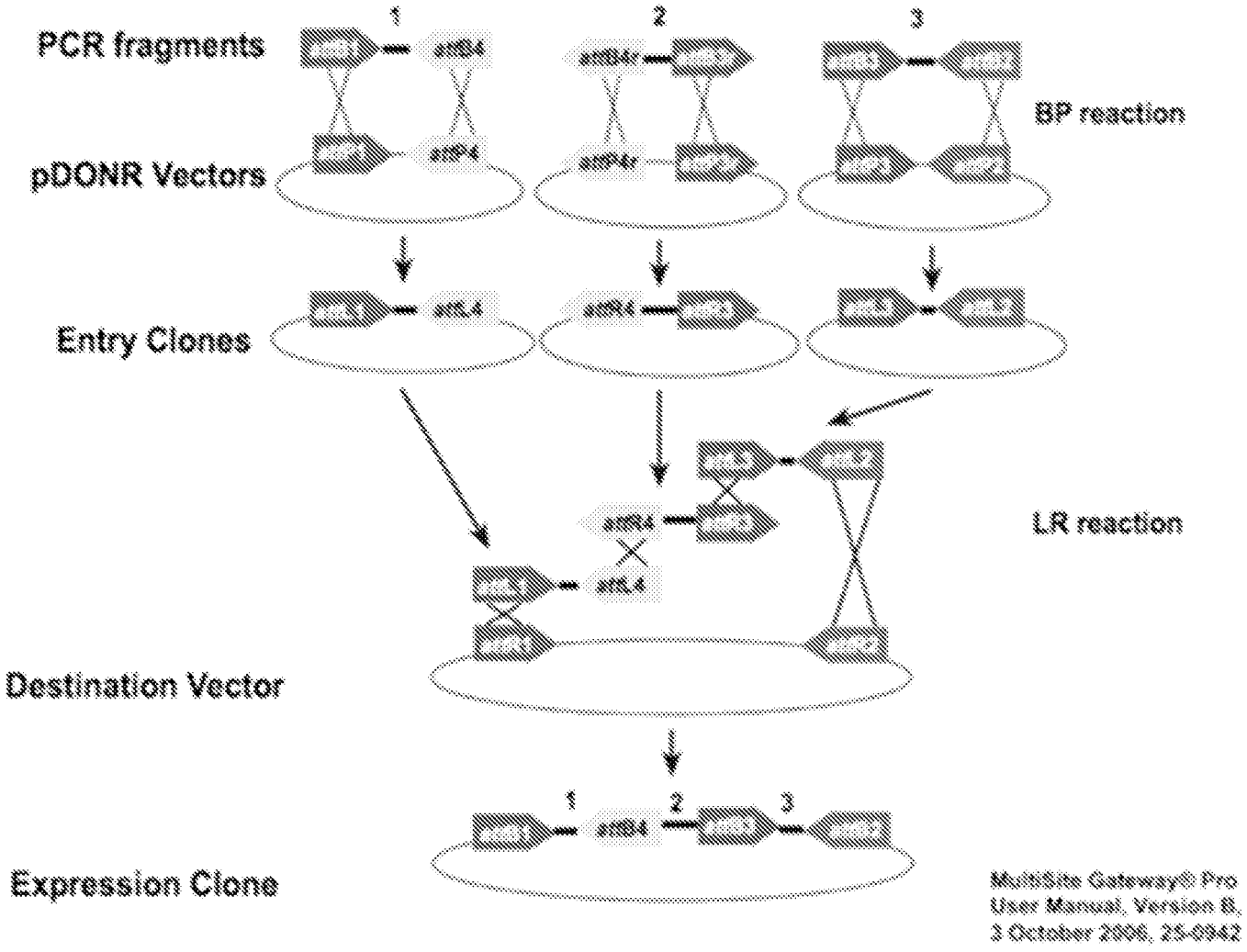
Key:

B1__*__B2 + P1_____P2

L1__*__L2 + R1_____R2

http://wolfson.huji.ac.il/expression/gatewayman.pdf

Figure 10



MultiSite Gateway® Pro User Manual, Version B, 3 October 2006, 25-0942

Figure 11

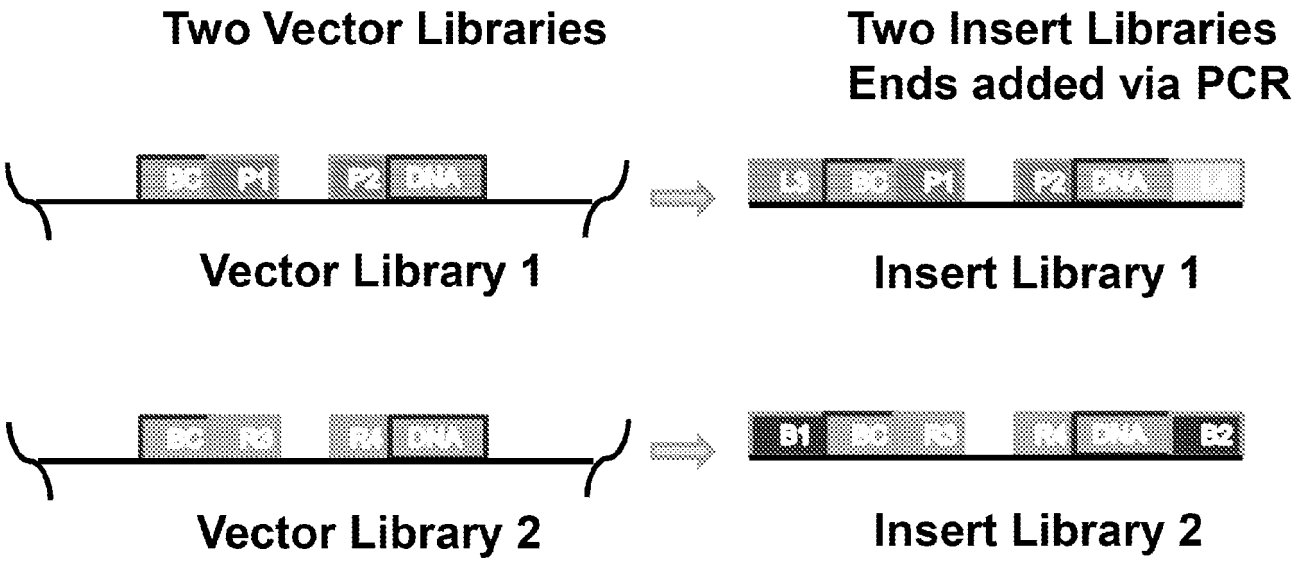

Two Vector Libraries
Two Insert Libraries
Ends added via PCR
P1
P2
DNA
Vector Library 1
Insert Library 1
B1
B2
Vector Library 2
Insert Library 2

Figure 12

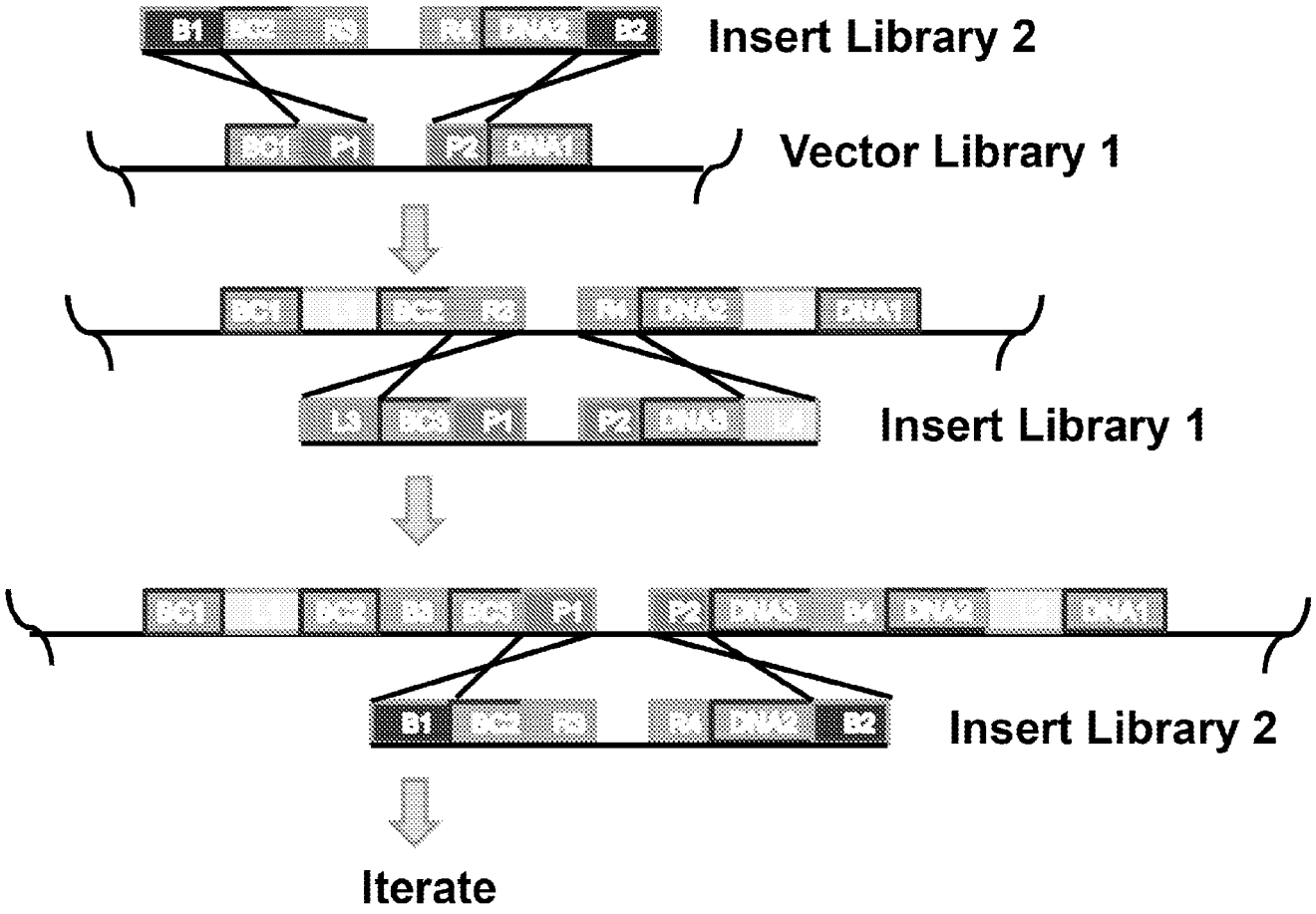

Insert Library 2
Vector Library 1
Insert Library 1
Insert Library 2
Iterate

Figure 13

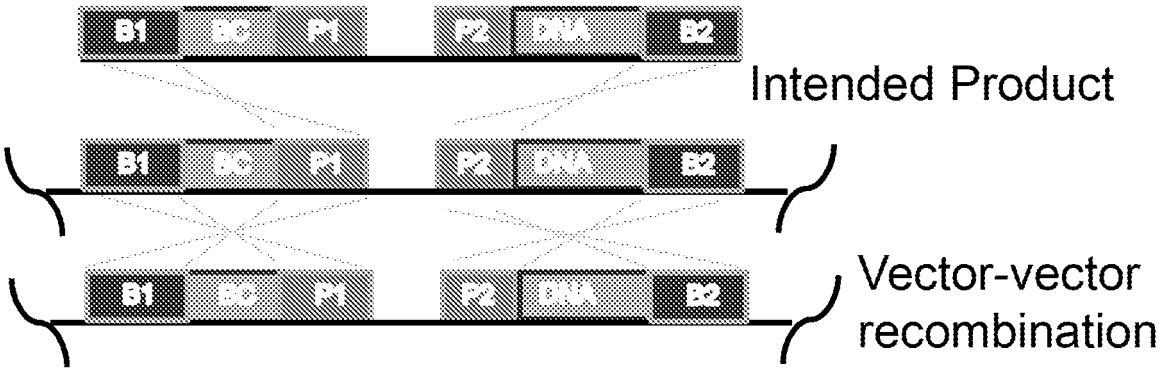

B1
P1
P2
DNA
B2
Intended Product
Vector-vector
recombination

Figure 14

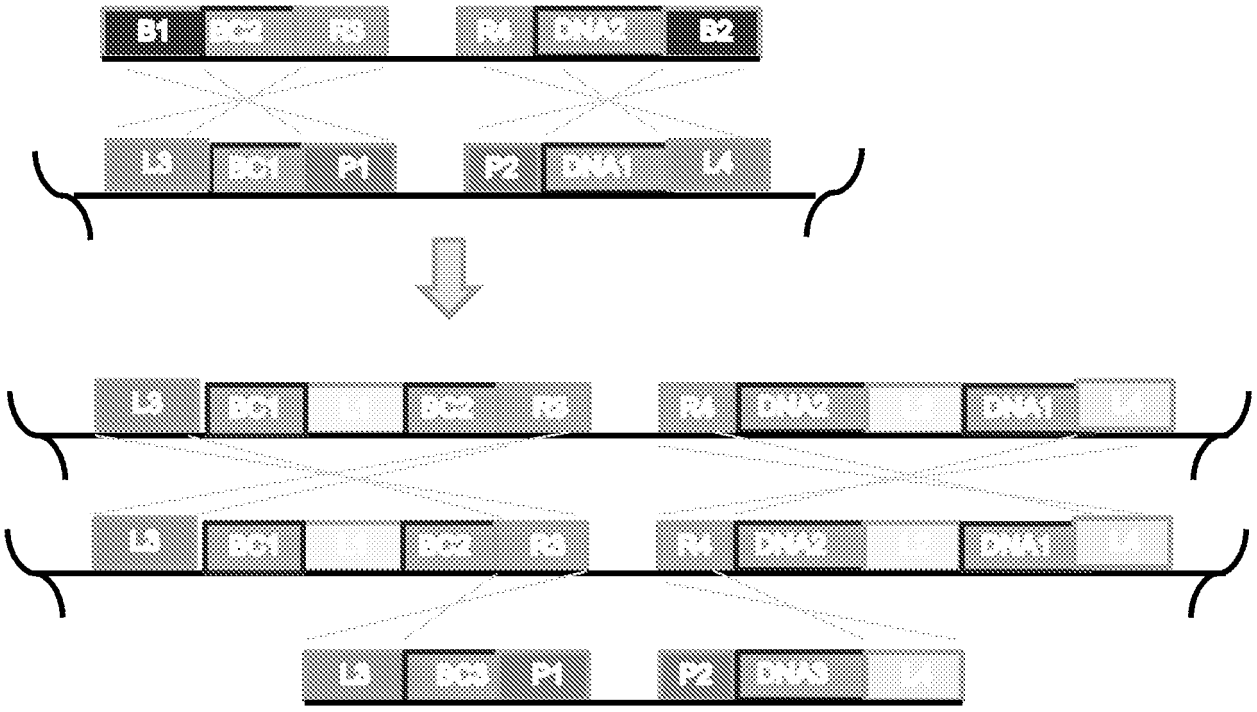


Figure 15

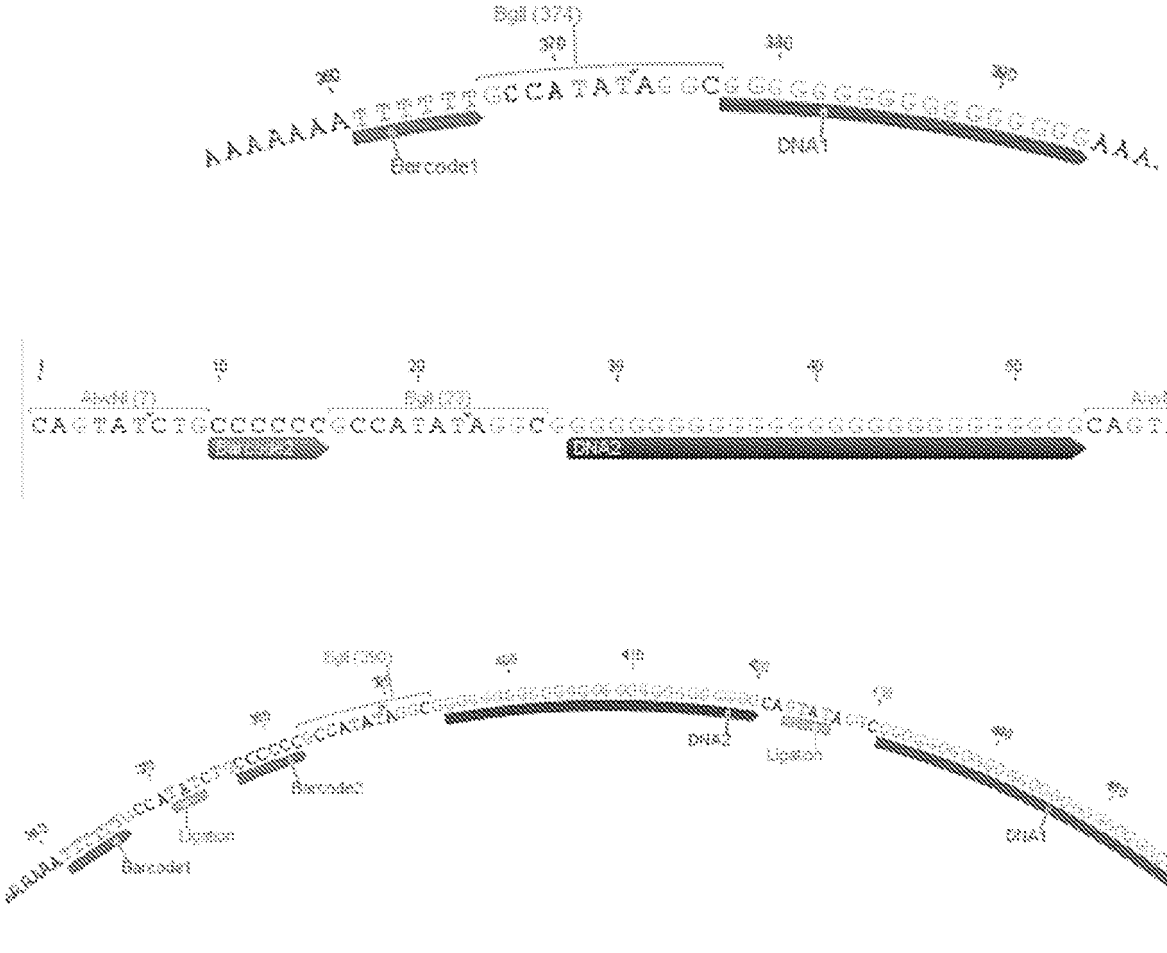

BglII (974)
Barcode1
DNA1
AhdI (1)
BglII (72)
AhdI (95)
Barcode2
DNA2
Ligation
Barcode1
Ligation
DNA1

Figure 16

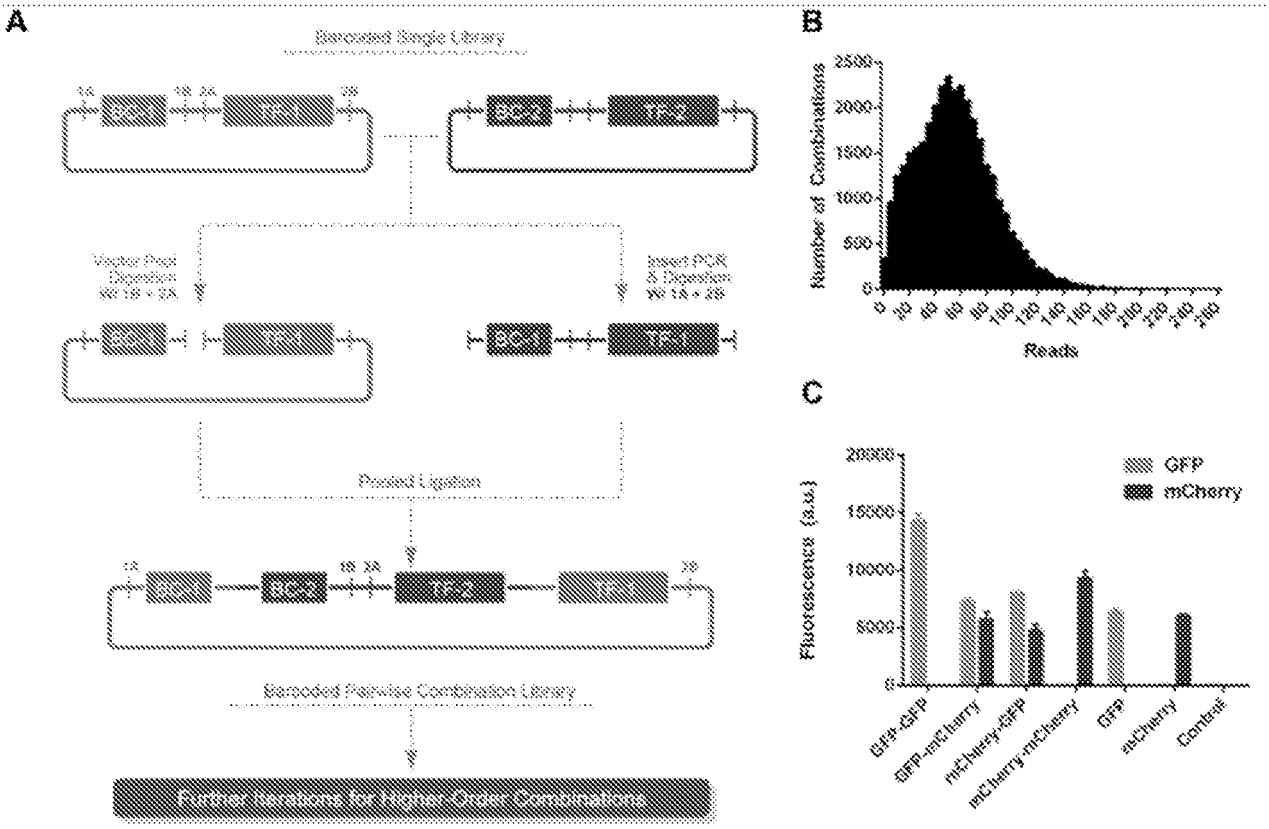

A
Barcoded Single Library
Vector Pool Digestion
Insert PCR & Digestion
Paired Ligation
Barcoded Pairwise Combination Library
Further Iterations for Higher Order Combinations
B
Number of Combinations
Reads
C
Fluorescence (a.u.)
GFP
mCherry
GFP-GFP
GFP-mCherry
mCherry-GFP
mCherry-mCherry
GFP
mCherry
Control

Figure 17

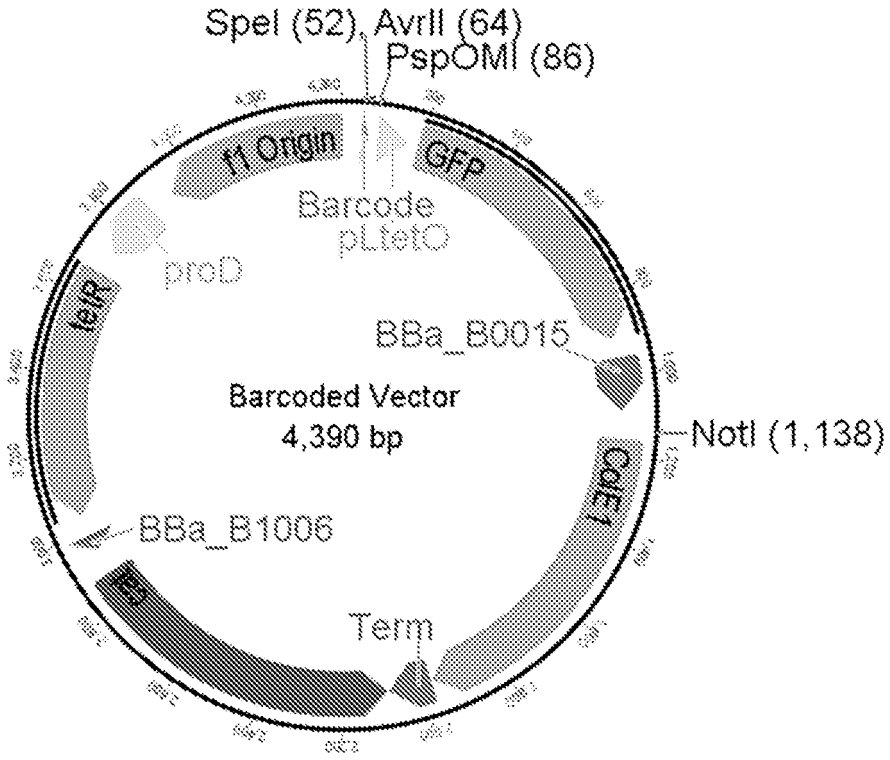

SpeI (52)
AvrII (64)
PspOMI (86)
f1 Origin
GFP
Barcode
pLtetO
proD
tetR
BBa_B0015
Barcoded Vector
4,390 bp
NotI (1,138)
CmR
ColE1
BBa_B1006
Term

Figure 18

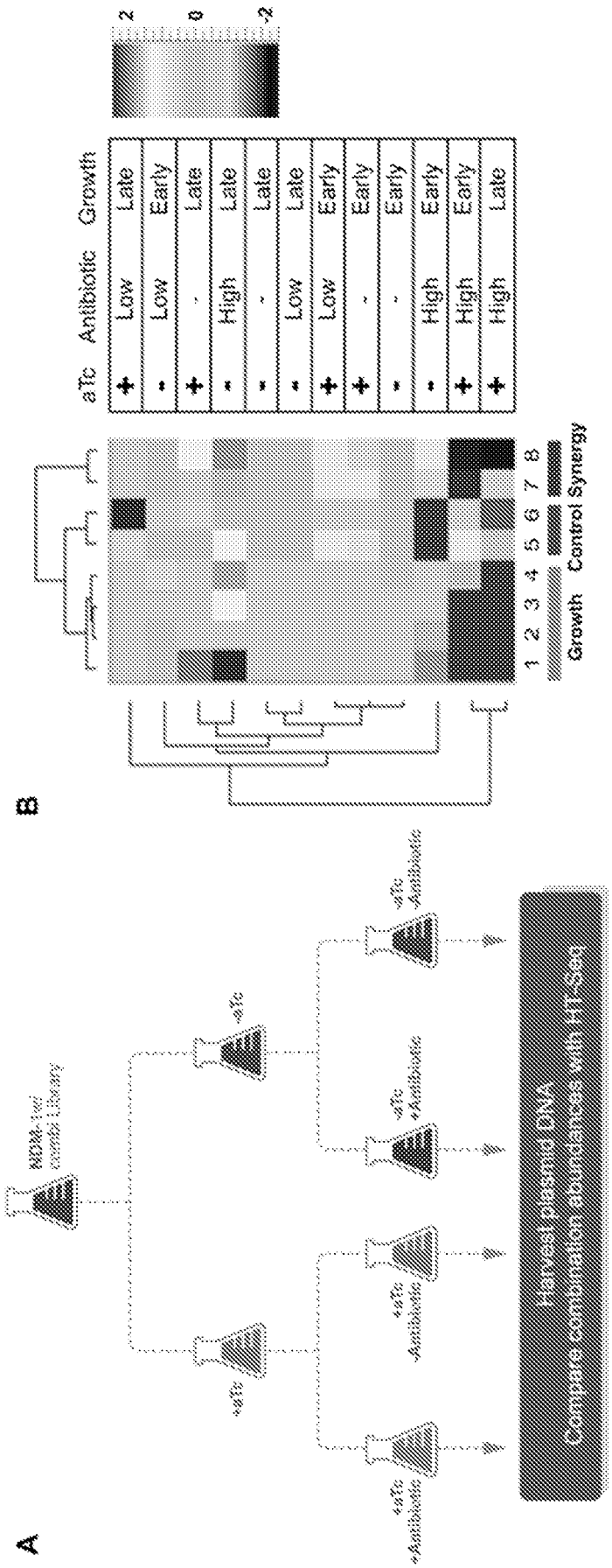

A
MDM-Tet comb Library
+aTc
+aTc
+aTc
+aTc
+aTc +Antibiotic
+aTc +Antibiotic
+aTc +Antibiotic
+aTc +Antibiotic
Harvest plasmid DNA
Compare counts/abundance with T=0
B
aTc
Antibiotic
Growth
+ Low Late
- Low Early
+ - Late
+ High Late
- - Late
- Low Late
+ Low Early
+ - Early
- - Early
- High Early
+ High Early
+ High Late
2
0
-2
1 2 3 4 5 6 7 8
Growth Control Synergy

Figure 19

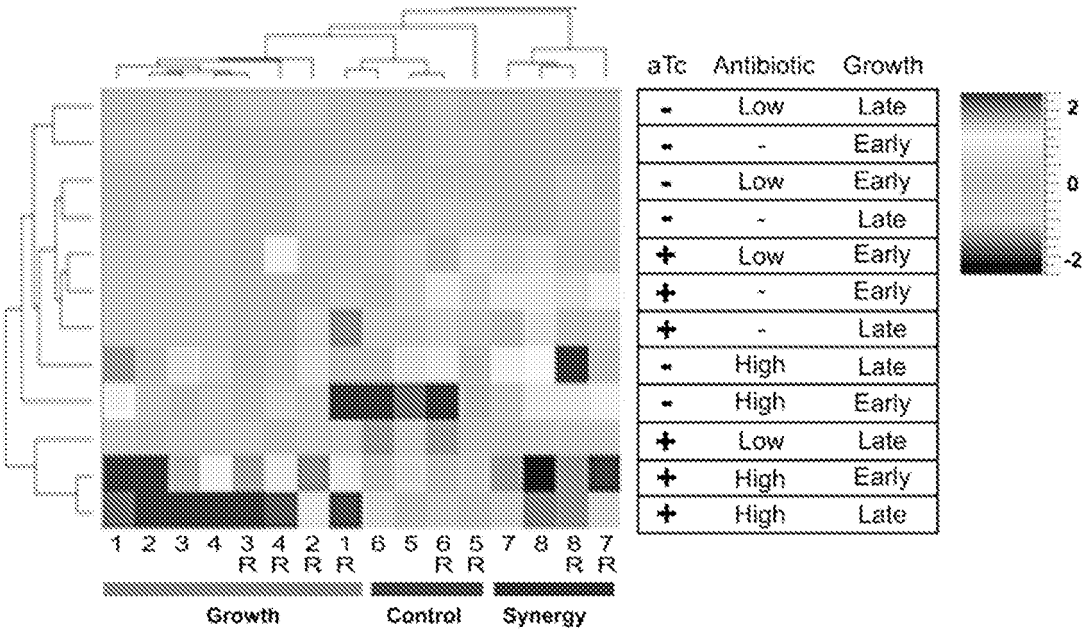

aTc
Antibiotic
Growth
- Low Late
- - Early
- Low Early
- - Late
+ Low Early
+ - Early
+ - Late
- High Late
- High Early
+ Low Late
+ High Early
+ High Late
2
0
-2
1 2 3 4 3R 4R 2R 1R 6 5 6R 5R 7 8 8R 7R
Growth
Control
Synergy

Figure 20

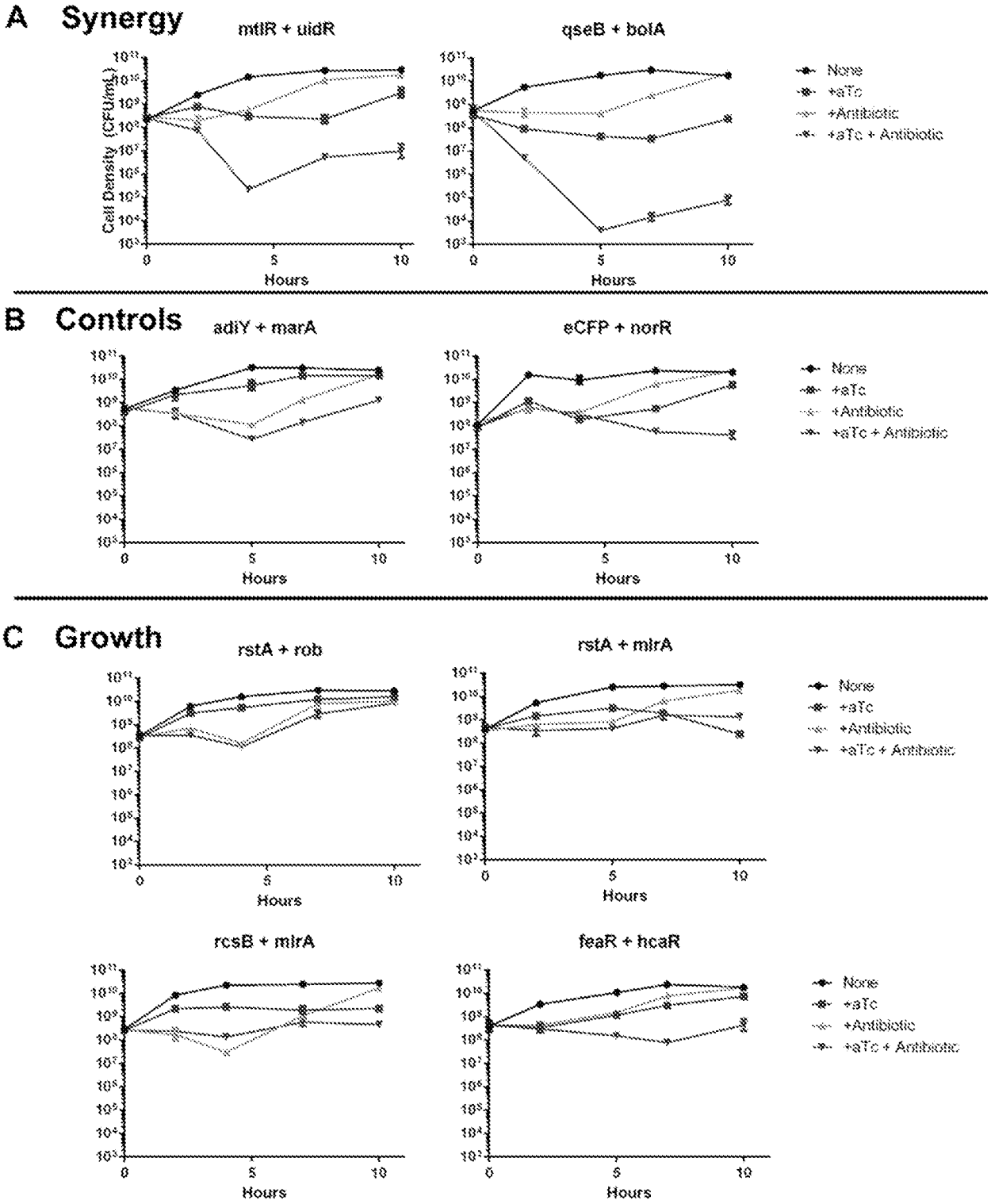

A Synergy
mtlR + uidR
qseB + bolA
Cell Density (CFU/mL)
Hours
None
+aTc
+Antibiotic
+aTc + Antibiotic
B Controls
adiY + marA
eCFP + norR
C Growth
rstA + rob
rstA + mlrA
rcsB + mlrA
feaR + hcaR

Figure 21

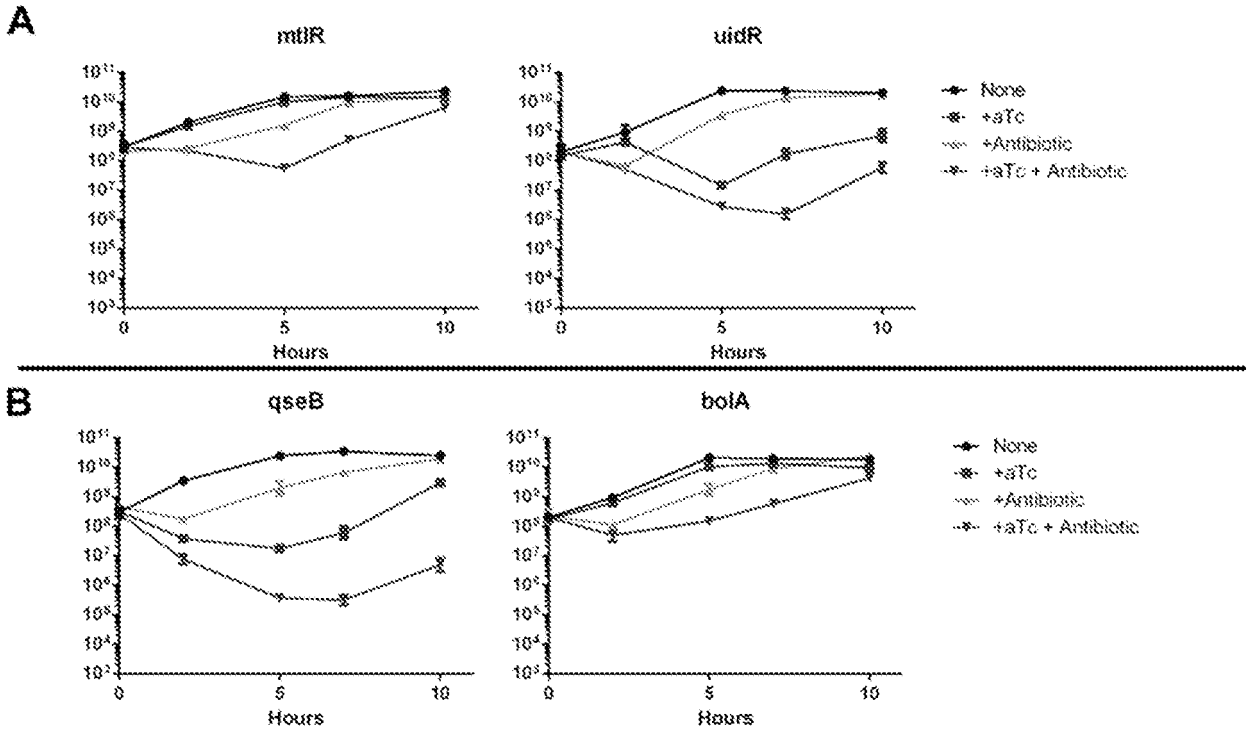

A
mtlR
uidR
None
+aTc
+Antibiotic
+aTc + Antibiotic
Hours
B
qseB
bolA
None
+aTc
+Antibiotic
+aTc + Antibiotic
Hours

Figure 22

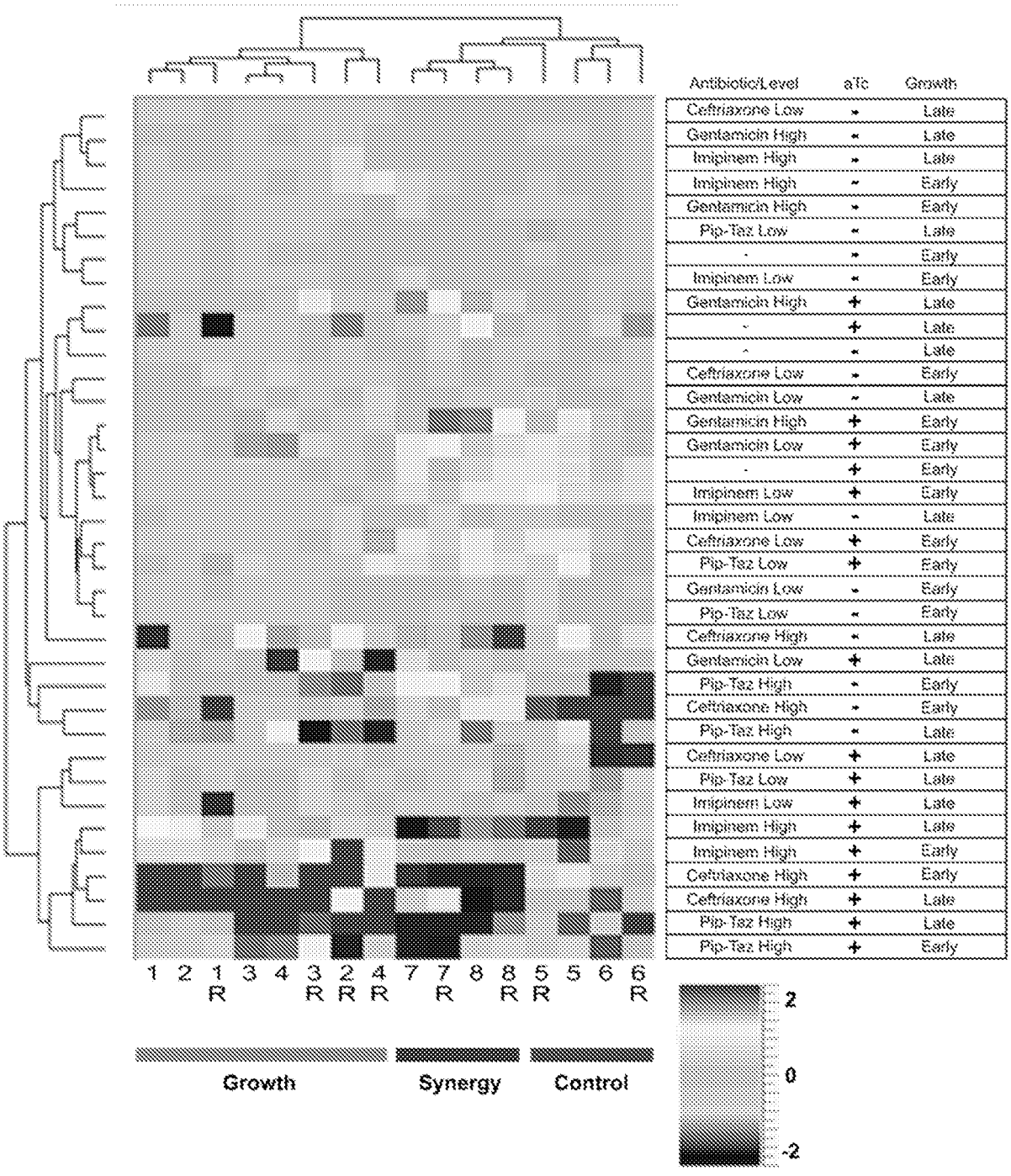

Antibiotic/Level aTc Growth
Ceftriaxone Low - Late
Gentamicin High - Late
Imipinem High - Late
Imipinem High - Early
Gentamicin High - Early
Pip-Taz Low - Late
- - Early
Imipinem Low - Early
Gentamicin High + Late
- + Late
- - Late
Ceftriaxone Low - Early
Gentamicin Low - Late
Gentamicin High + Early
Gentamicin Low + Early
- + Early
Imipinem Low + Early
Imipinem Low - Late
Ceftriaxone Low + Early
Pip-Taz Low + Early
Gentamicin Low - Early
Pip-Taz Low - Early
Ceftriaxone High - Late
Gentamicin Low + Late
Pip-Taz High - Early
Ceftriaxone High - Early
Pip-Taz High - Late
Ceftriaxone Low + Late
Pip-Taz Low + Late
Imipinem Low + Late
Imipinem High + Late
Imipinem High + Early
Ceftriaxone High + Early
Ceftriaxone High + Late
Pip-Taz High + Late
Pip-Taz High + Early
1 2 1R 3 4 3R 2R 4R 7 7R 8 8R 5R 5 6 6R
Growth
Synergy
Control
2
0
-2

Figure 23

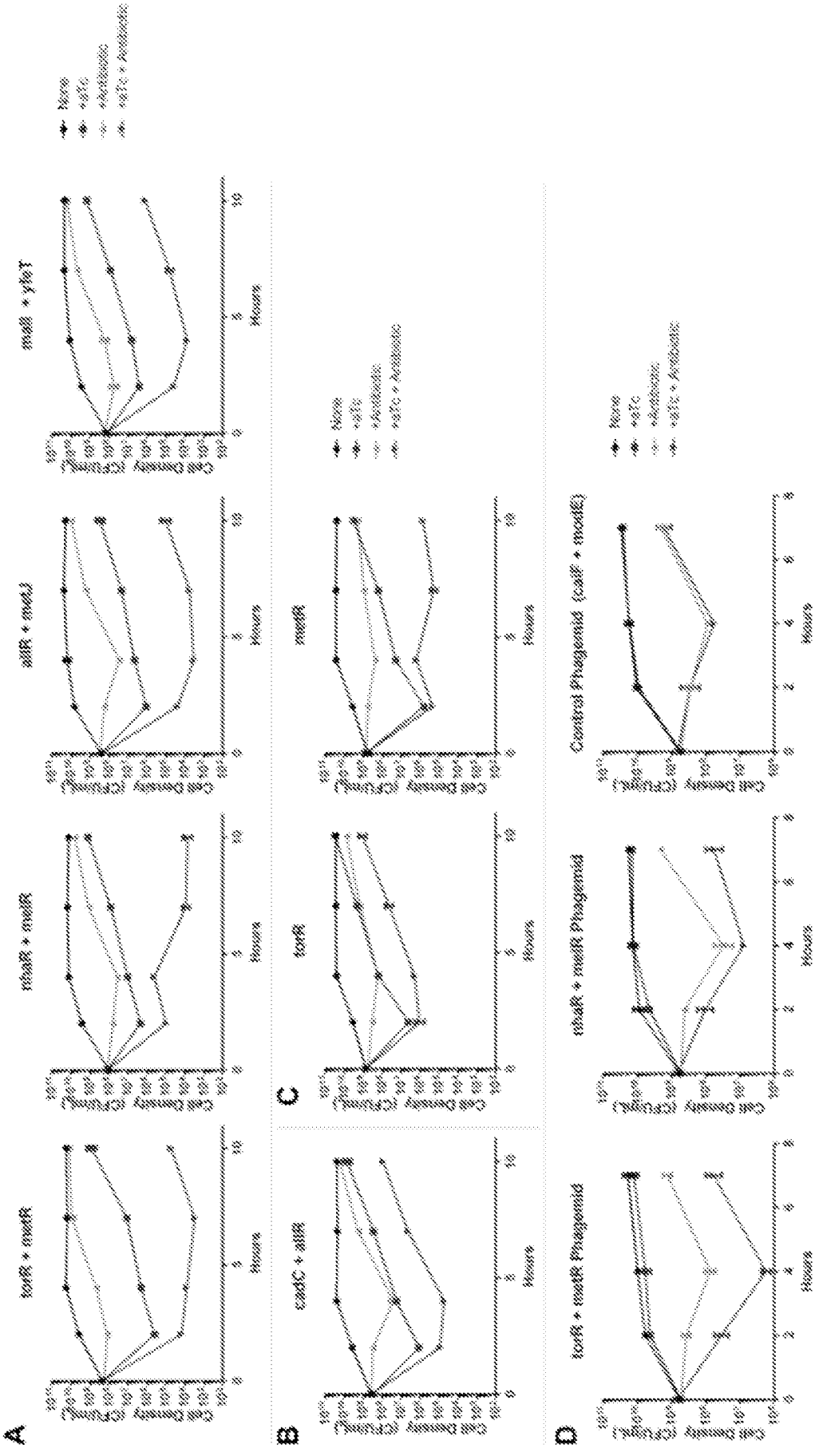

A
B
C
D
tetR + mefR
cadC + ampR
ribaR + mefR
torR
ampR + tetT
ampR + mefA
mefR
tetR + mefR Plasmid
Control Plasmid (cadF + mefE)
None
+otc
+Antibiotic
+otc + Antibiotic
Cell Density (CFU/mL)
Hours

Figure 24

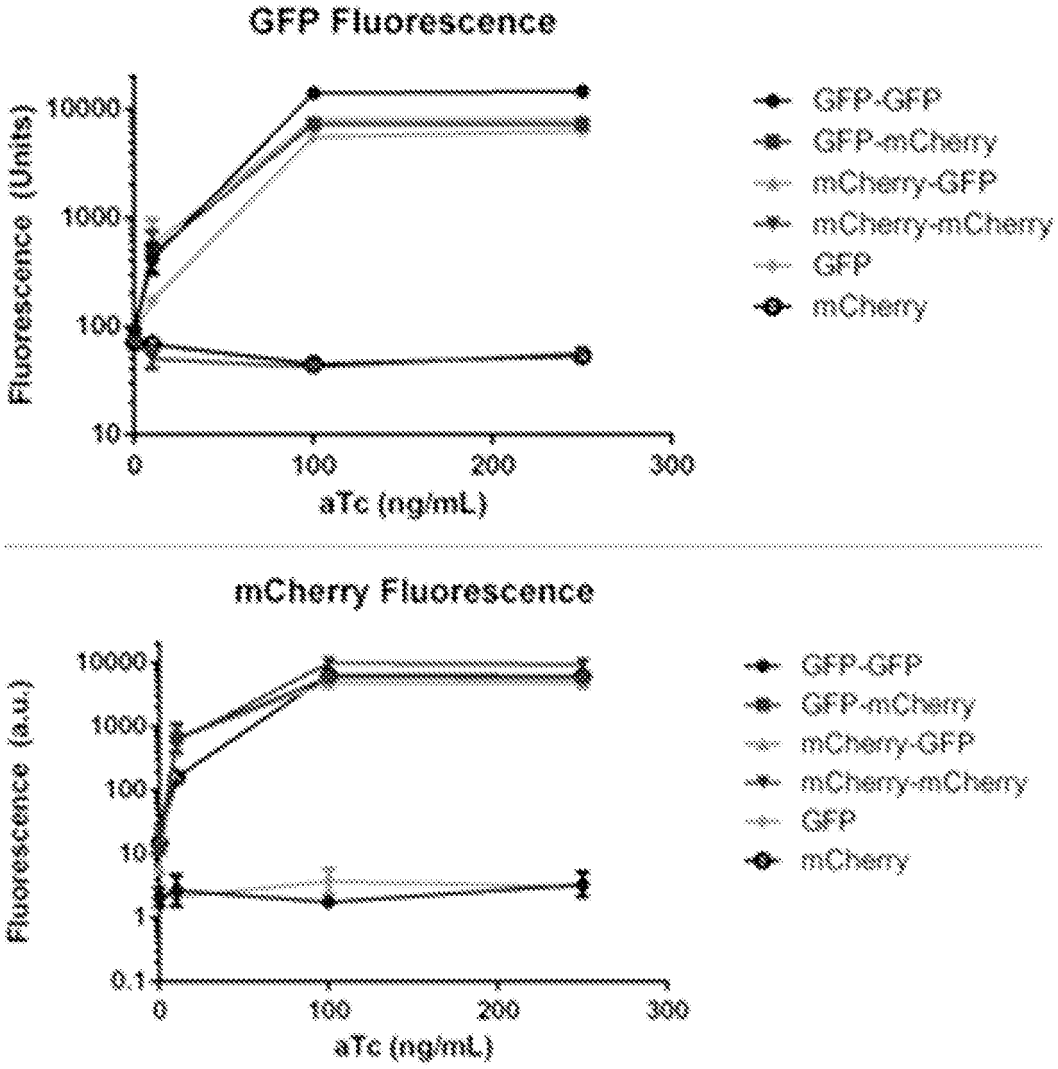

GFP Fluorescence
Fluorescence (Units)
10000
1000
100
10
0
100
200
300
aTc (ng/mL)
GFP-GFP
GFP-mCherry
mCherry-GFP
mCherry-mCherry
GFP
mCherry
mCherry Fluorescence
Fluorescence (a.u.)
10000
1000
100
10
1
0.1
0
100
200
300
aTc (ng/mL)
GFP-GFP
GFP-mCherry
mCherry-GFP
mCherry-mCherry
GFP
mCherry

Figure 25

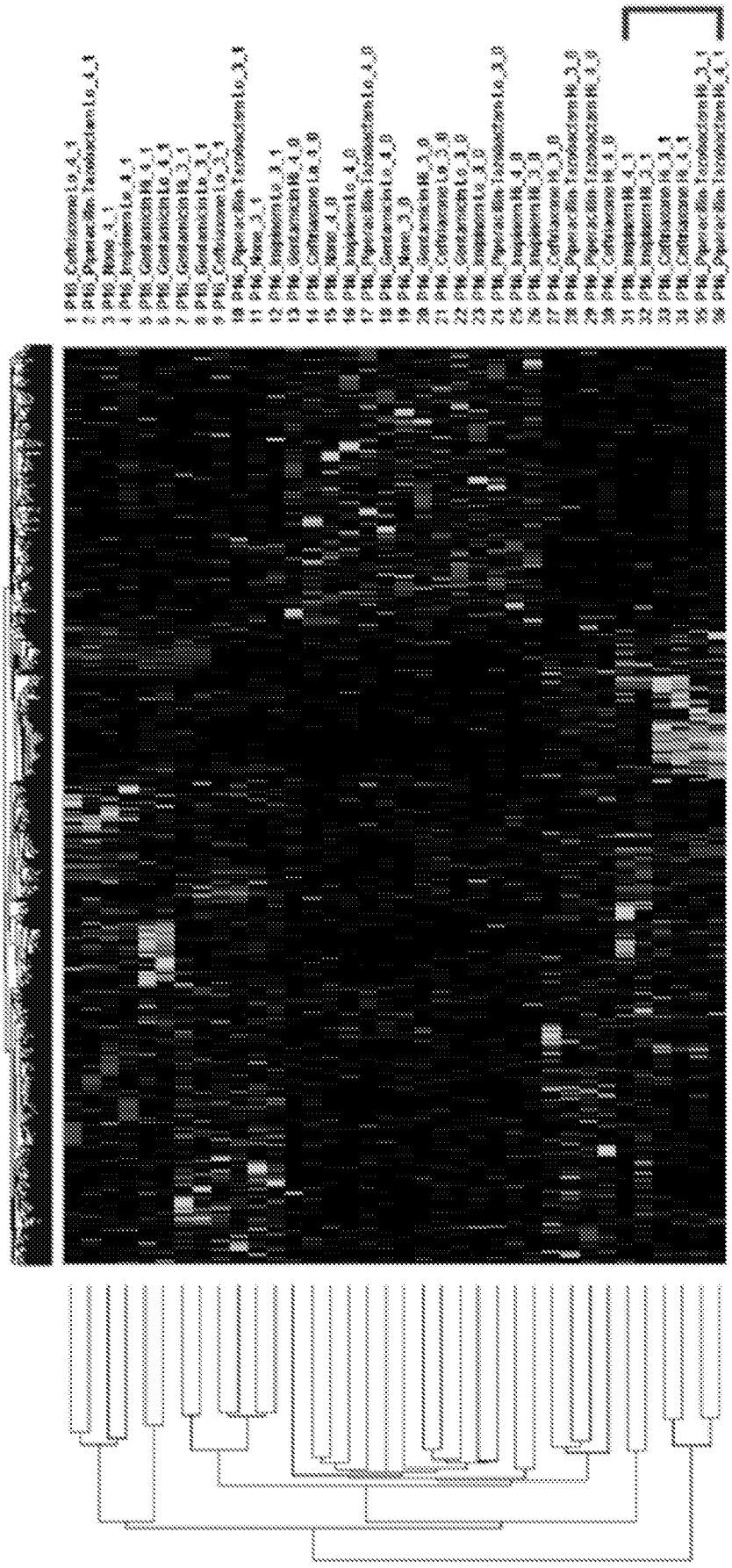


Figure 26

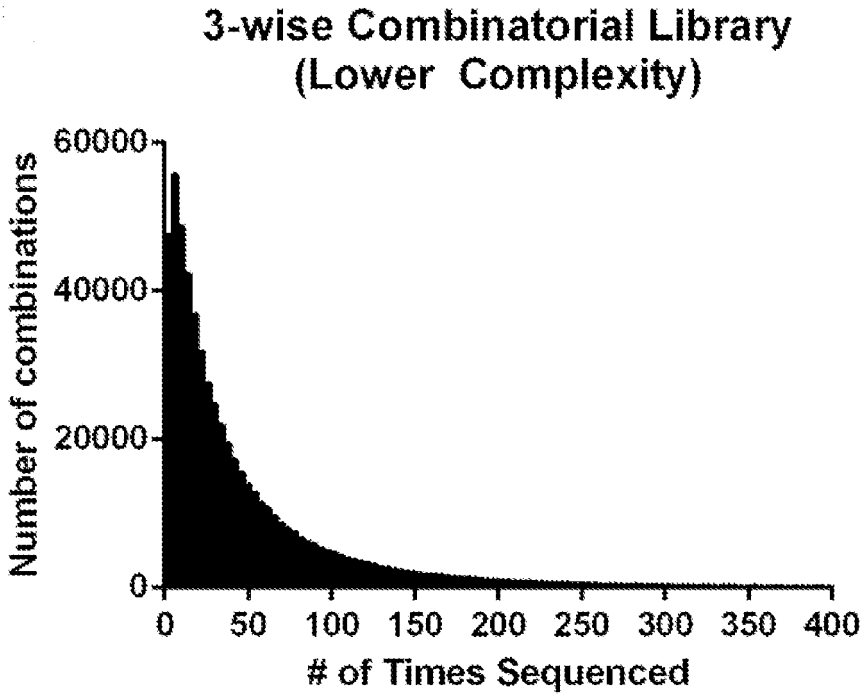


Figure 27

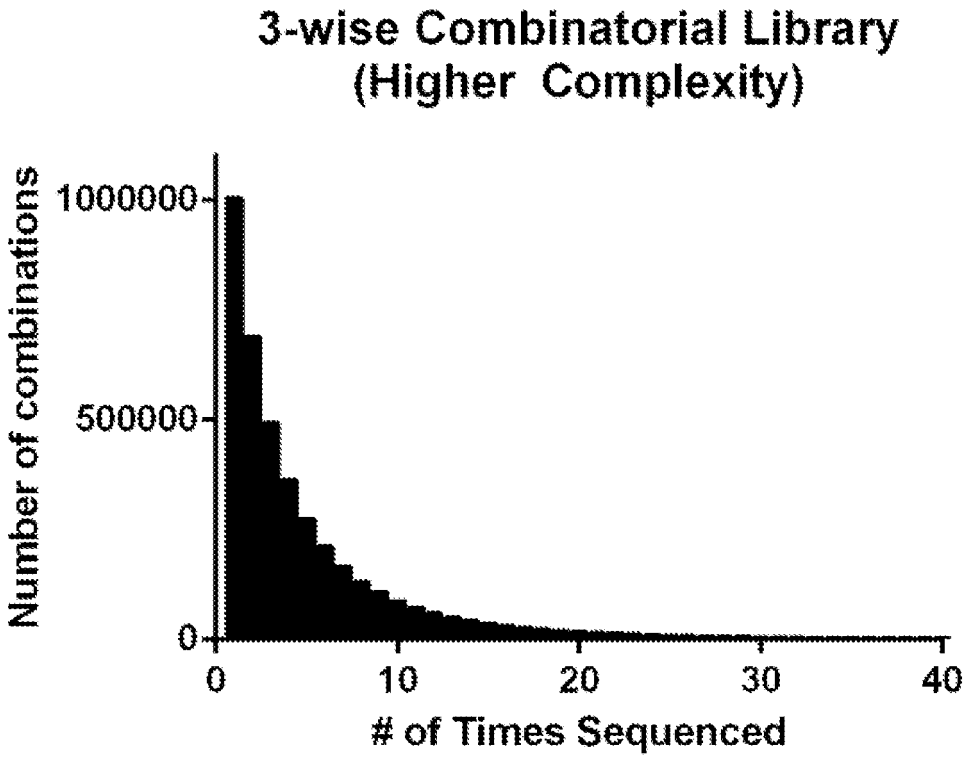
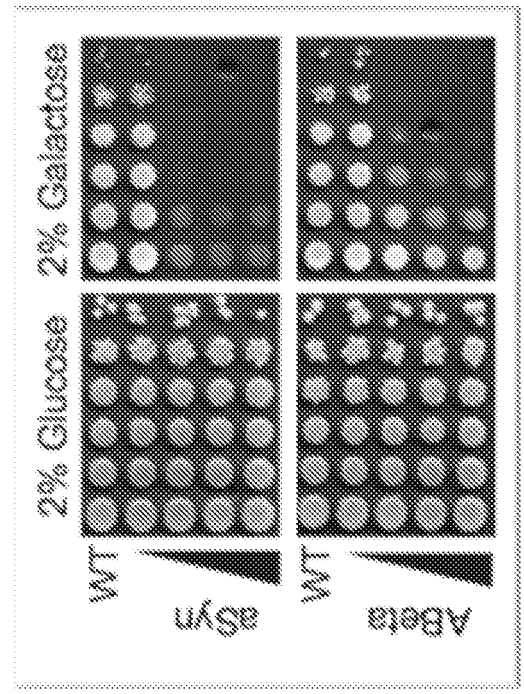


Figure 28


2% Glucose
2% Galactose
WT
aSyn
WT
Abeta

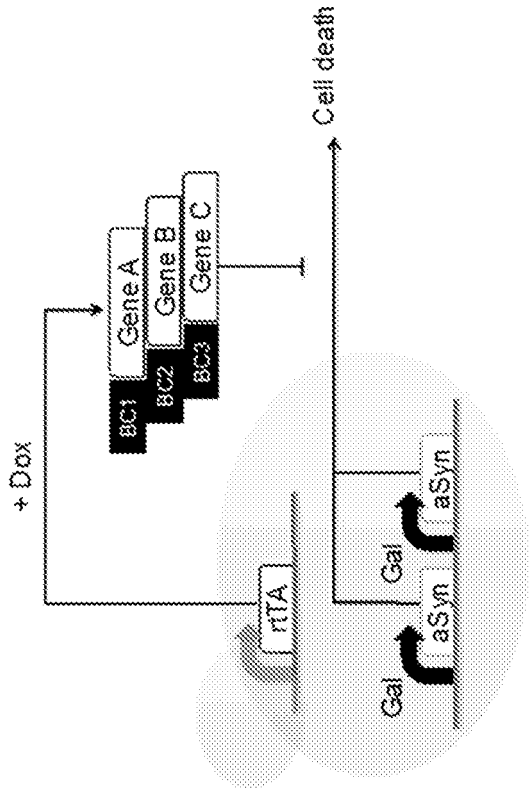

+ Dox
Gene A
Gene B
Gene C
BC1
BC2
BC3
rtTA
Gal
aSyn
Gal
aSyn
Cell death

Figure 29

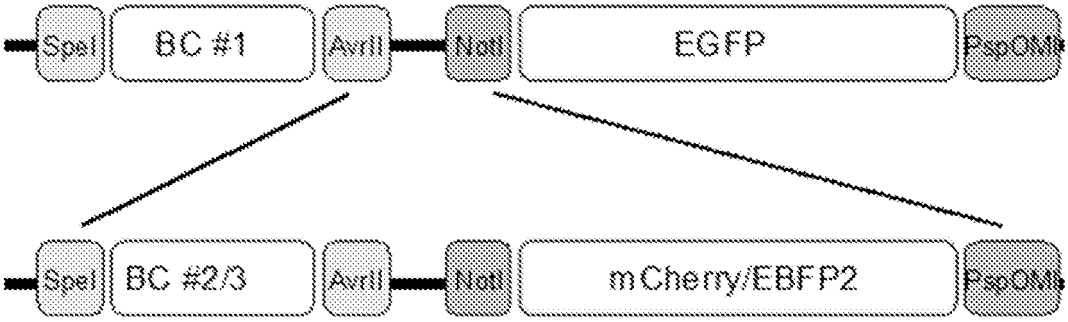


Figure 30

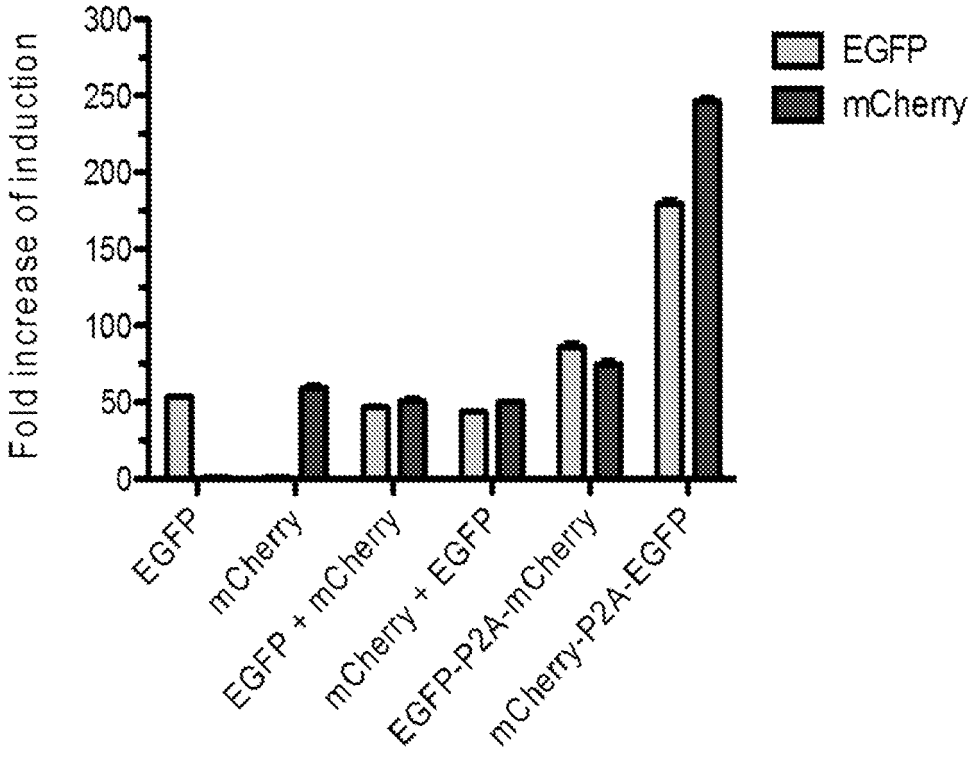


Figure 31

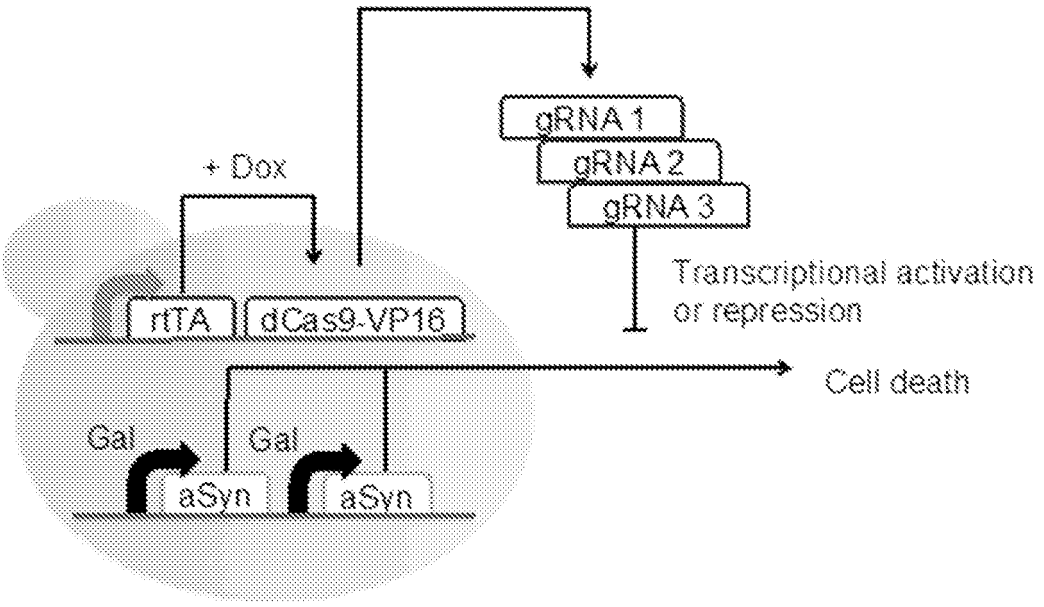


Figure 32

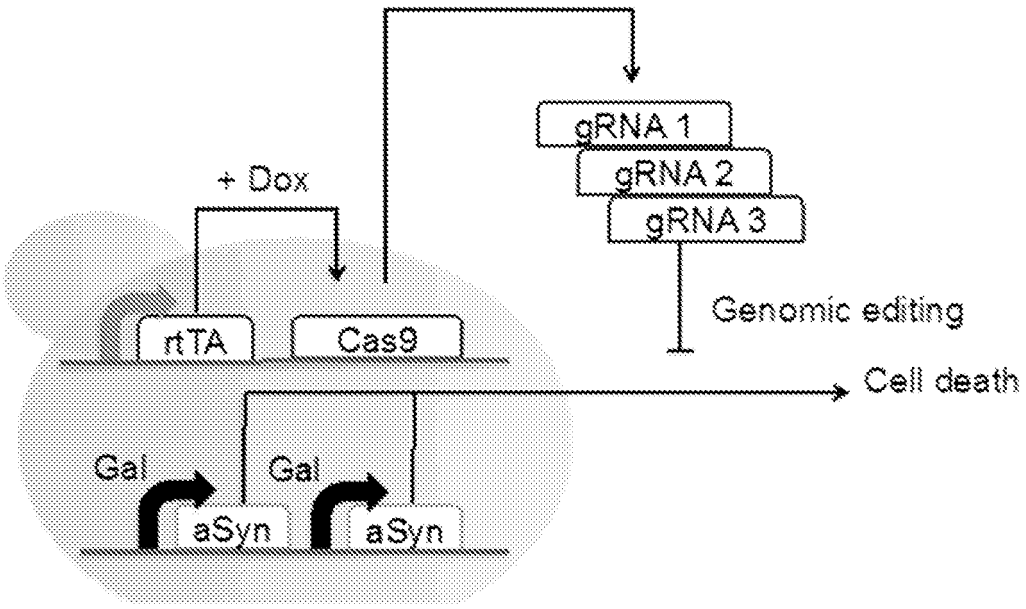


Figure 33

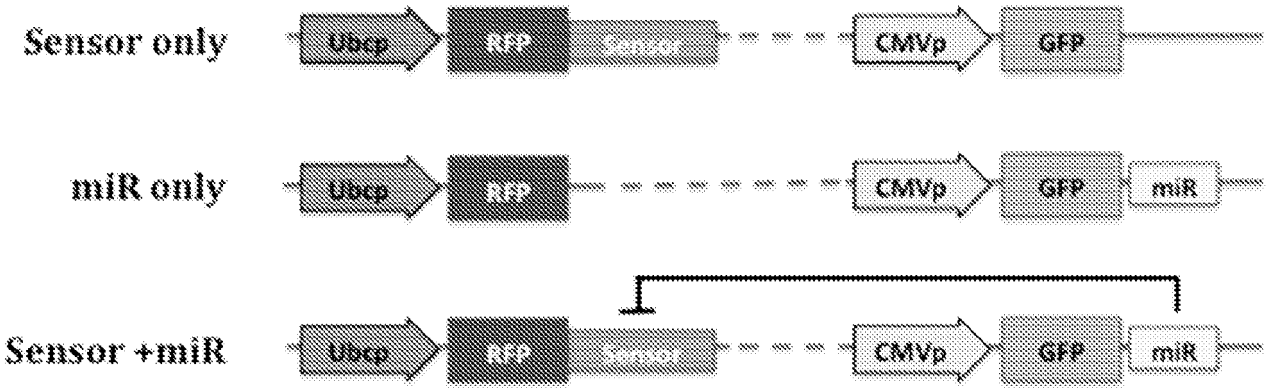

Sensor only
Ubcp
RFP
Sensor
CMVp
GFP
miR only
Ubcp
RFP
CMVp
GFP
miR
Sensor +miR
Ubcp
RFP
Sensor
CMVp
GFP
miR

Figure 34

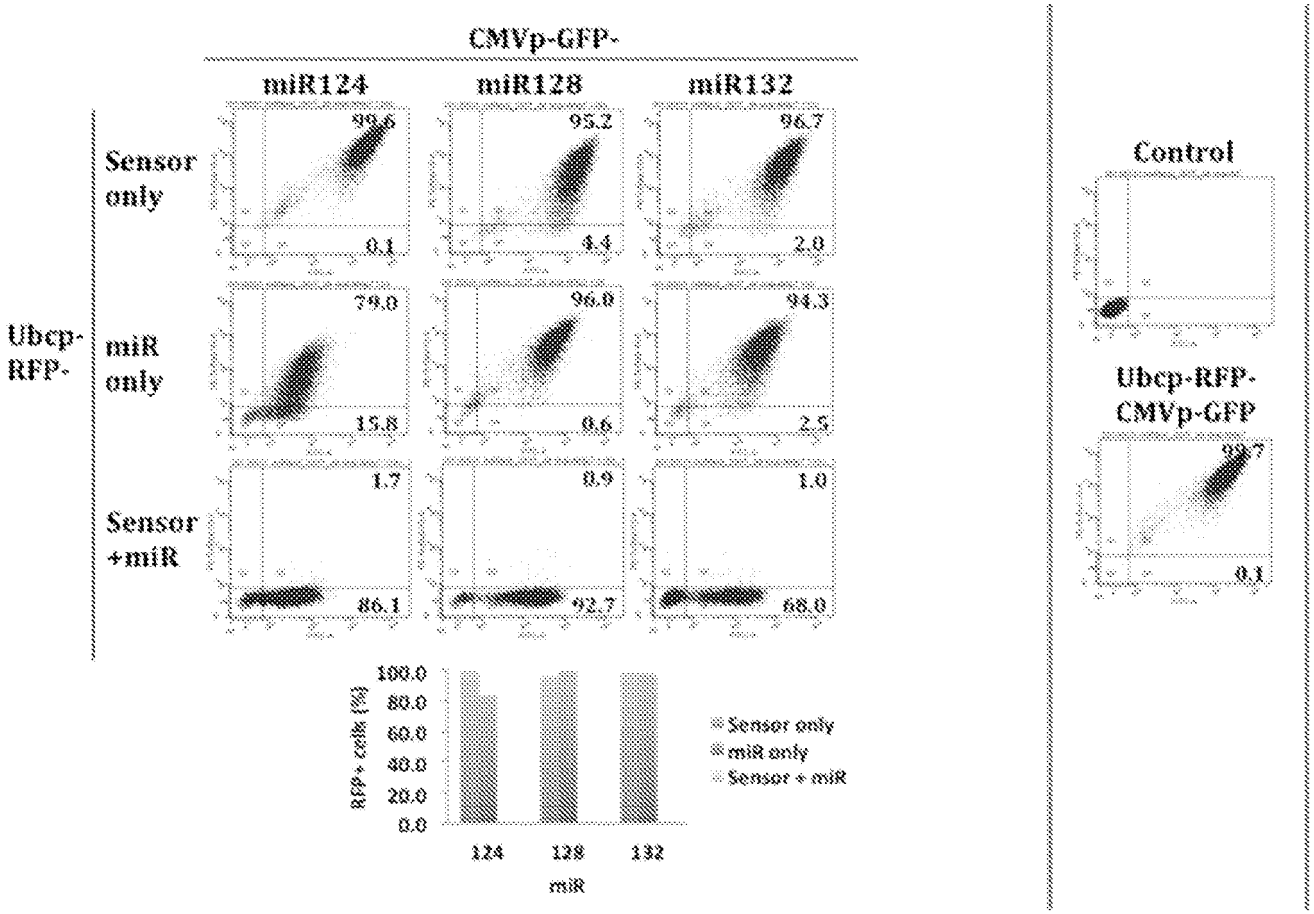

CMVp-GFP-
miR124
miR128
miR132
Ubcp-RFP-
Sensor only
miR only
Sensor +miR
99.6
0.1
95.2
4.4
96.7
2.0
79.0
15.8
96.0
0.6
94.3
2.5
1.7
86.1
8.9
92.7
1.0
68.0
RFP+ cells (%)
100.0
80.0
60.0
40.0
20.0
0.0
124
128
132
miR
Sensor only
miR only
Sensor + miR
Control
Ubcp-RFP-CMVp-GFP
99.7
0.1

Figure 35

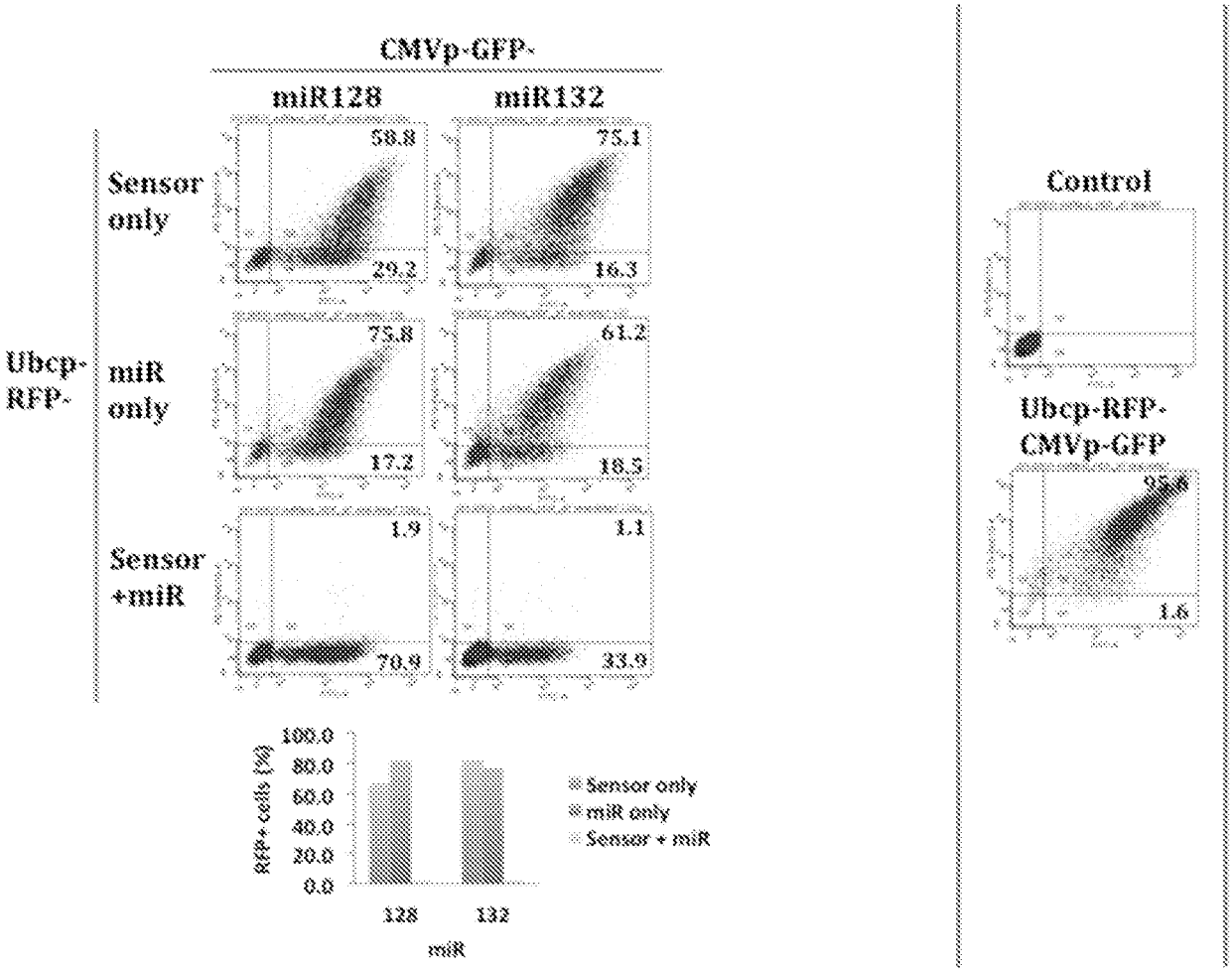

CMVp-GFP-
miR128
miR132
Ubcp-RFP-
Sensor only
miR only
Sensor +miR
58.8
29.2
75.1
16.3
75.8
17.2
61.2
18.5
1.9
70.9
1.1
33.9
RFP+ cells (%)
100.0
80.0
60.0
40.0
20.0
0.0
128
132
miR
Sensor only
miR only
Sensor + miR
Control
Ubcp-RFP-
CMVp-GFP
1.6

Figure 36

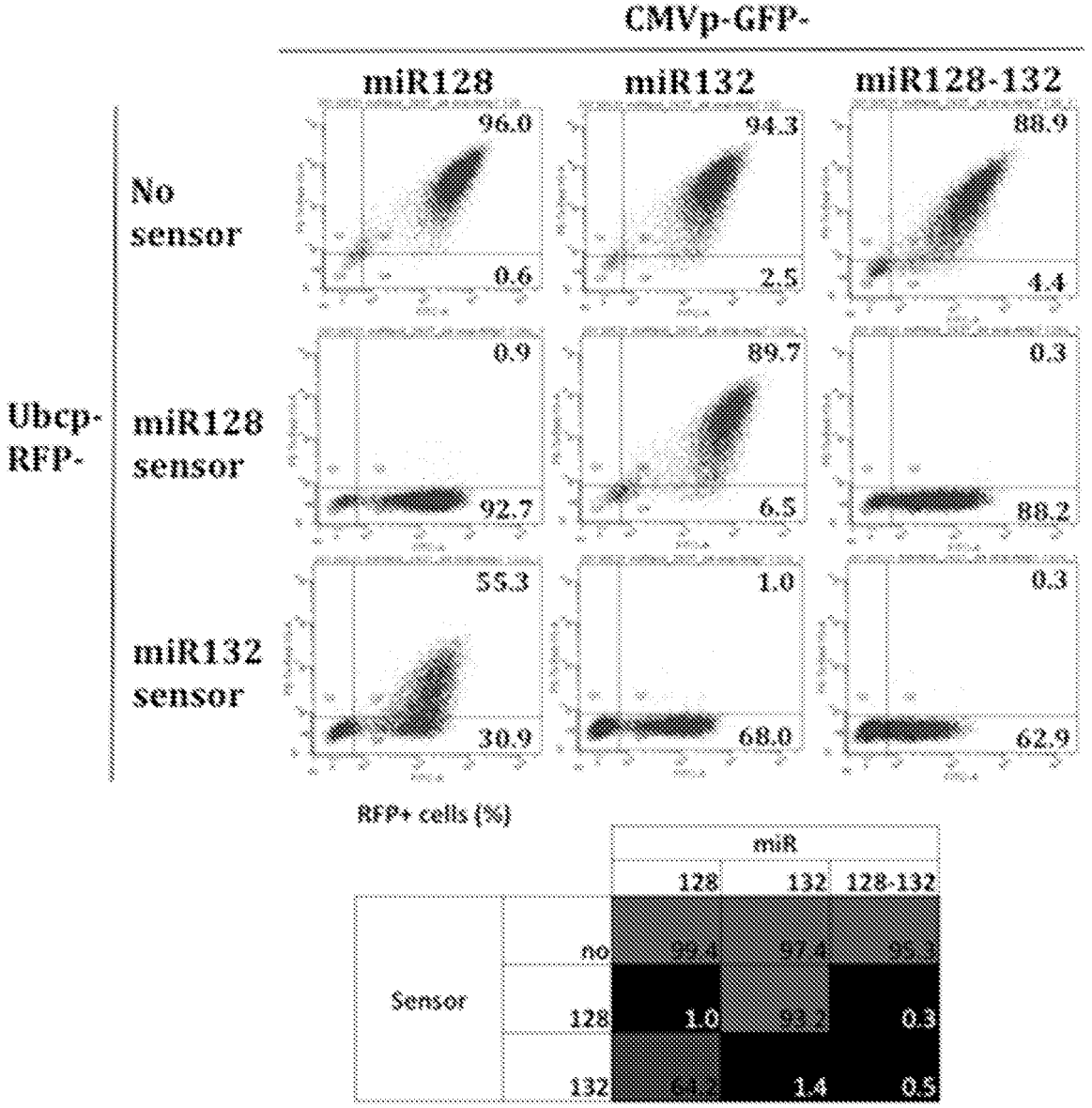

CMVp-GFP-
miR128
miR132
miR128-132
Ubcp-RFP-
No sensor
miR128 sensor
miR132 sensor
96.0
0.6
94.3
2.5
88.9
4.4
0.9
92.7
89.7
6.5
0.3
88.2
55.3
30.9
1.0
68.0
0.3
62.9
RFP+ cells (%)
miR
128
132
128-132
Sensor
no
128
132
1.0
0.3
1.4
0.5

Figure 37

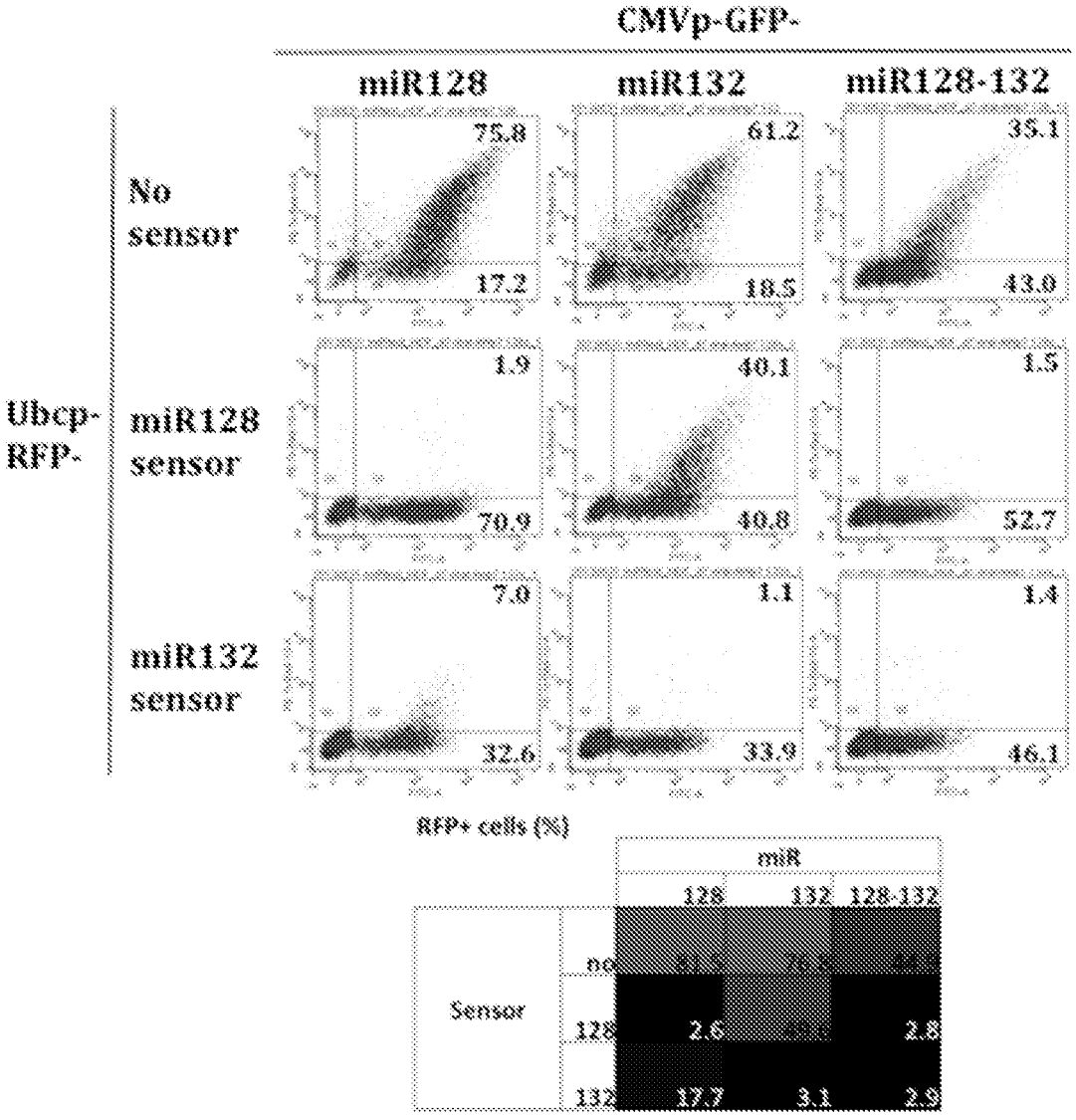
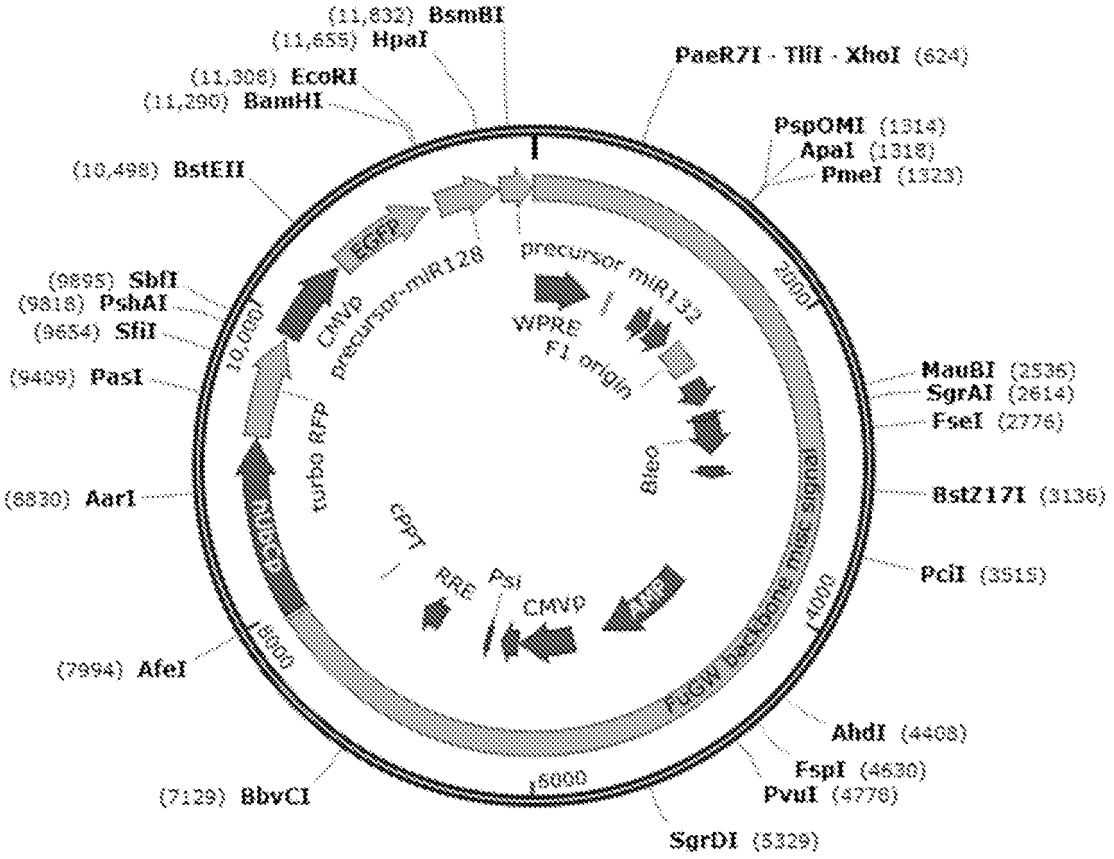

CMVp-GFP-
miR128
miR132
miR128-132
Ubcp-RFP-
No sensor
miR128 sensor
miR132 sensor
75.8
17.2
61.2
18.5
35.1
43.0
1.9
70.9
40.1
40.8
1.5
52.7
7.0
32.6
1.1
33.9
1.4
46.1
RFP+ cells (%)
miR
128
132
128-132
Sensor
no
128
132
2.6
17.7
3.1
2.8
2.9

Figure 38


(11,832) BsmBI
(11,655) HpaI
(11,306) EcoRI
(11,296) BamHI
(10,498) BstEII
(9895) SbfI
(9818) PshAI
(9654) SfiI
(9409) PasI
(6830) AarI
(7994) AfeI
(7129) BbvCI
PaeR7I - TliI - XhoI (624)
PspOMI (1314)
ApaI (1318)
PmeI (1323)
MauBI (2536)
SgrAI (2614)
FseI (2776)
BstZ17I (3136)
PciI (3515)
AhdI (4408)
FspI (4630)
PvuI (4778)
SgrDI (5329)
EGFP
CMVp
precursor-miR126
precursor miR132
WPRE
f1 origin
Bleo
turbo RFP
cPPT
RRE
Psi
CMVp
ΔLTR
pUC ORIGIN
2000
4000
6000
8000
10,000