Figure 1

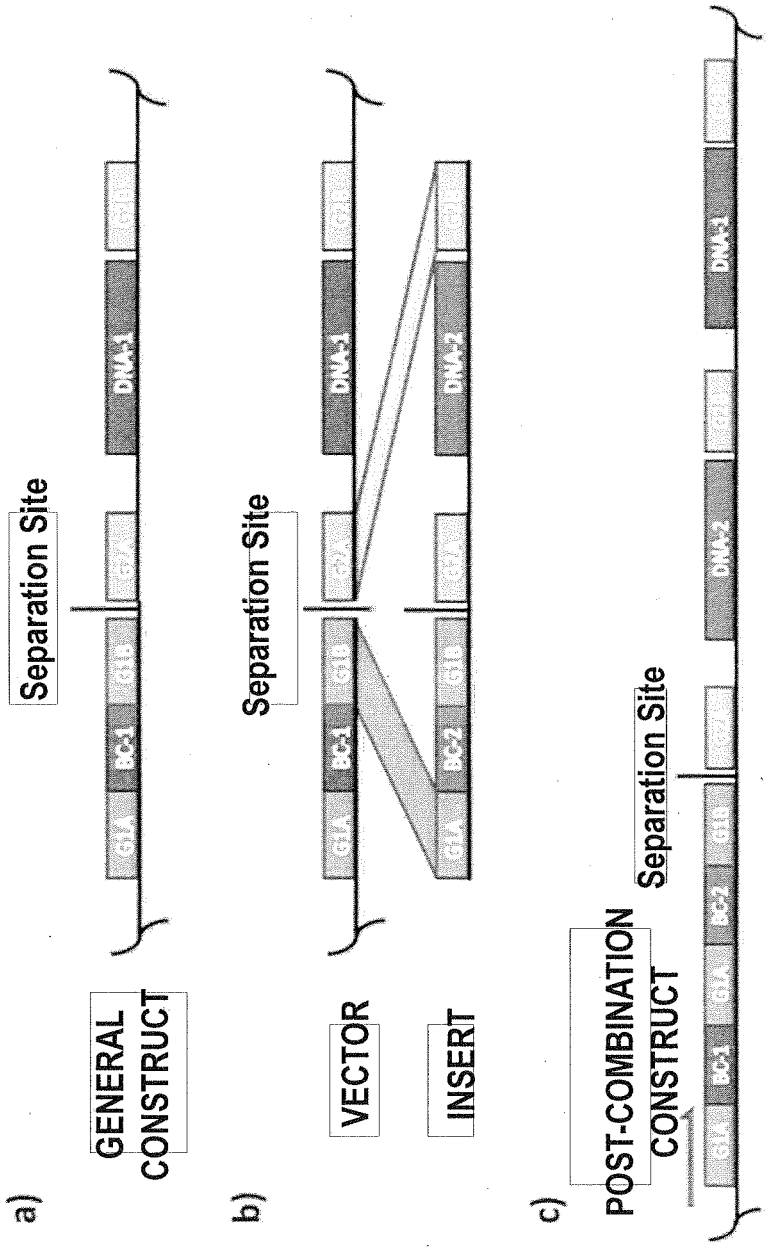
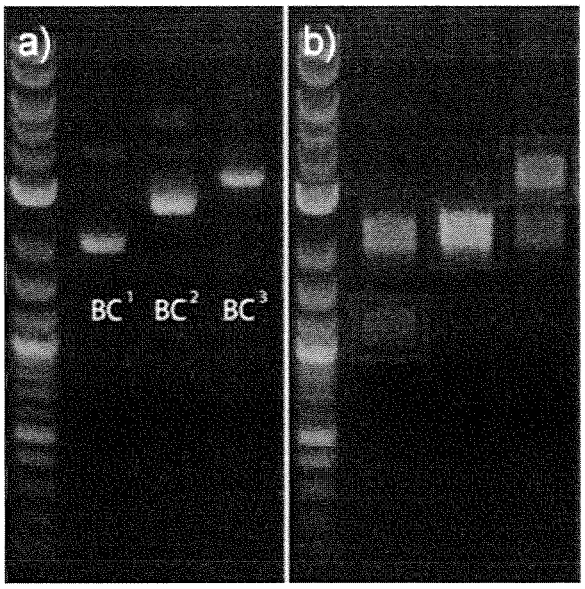

a)
GENERAL CONSTRUCT
Separation Site
b)
VECTOR
INSERT
Separation Site
c)
POST-COMBINATION CONSTRUCT
Separation Site

**Figure 2**



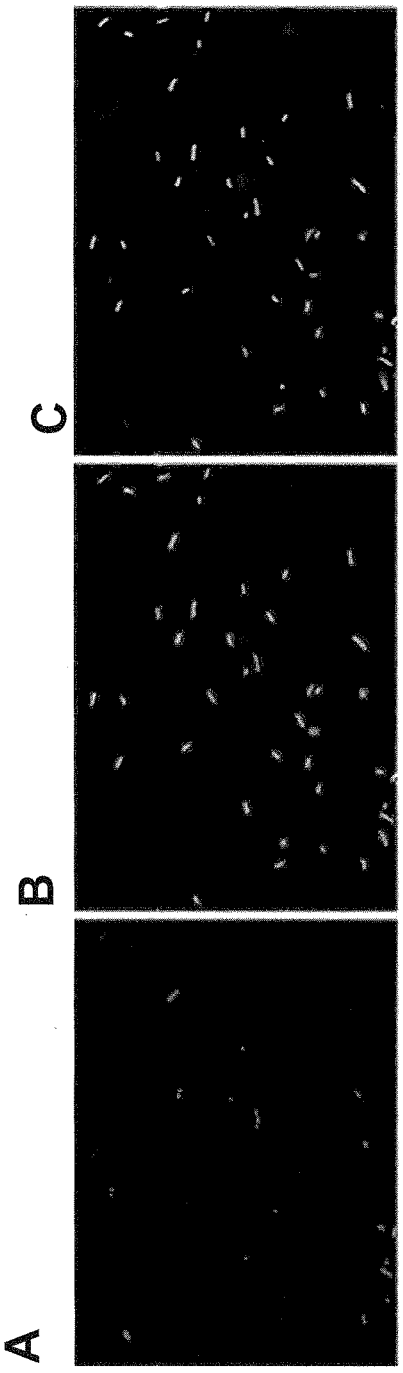
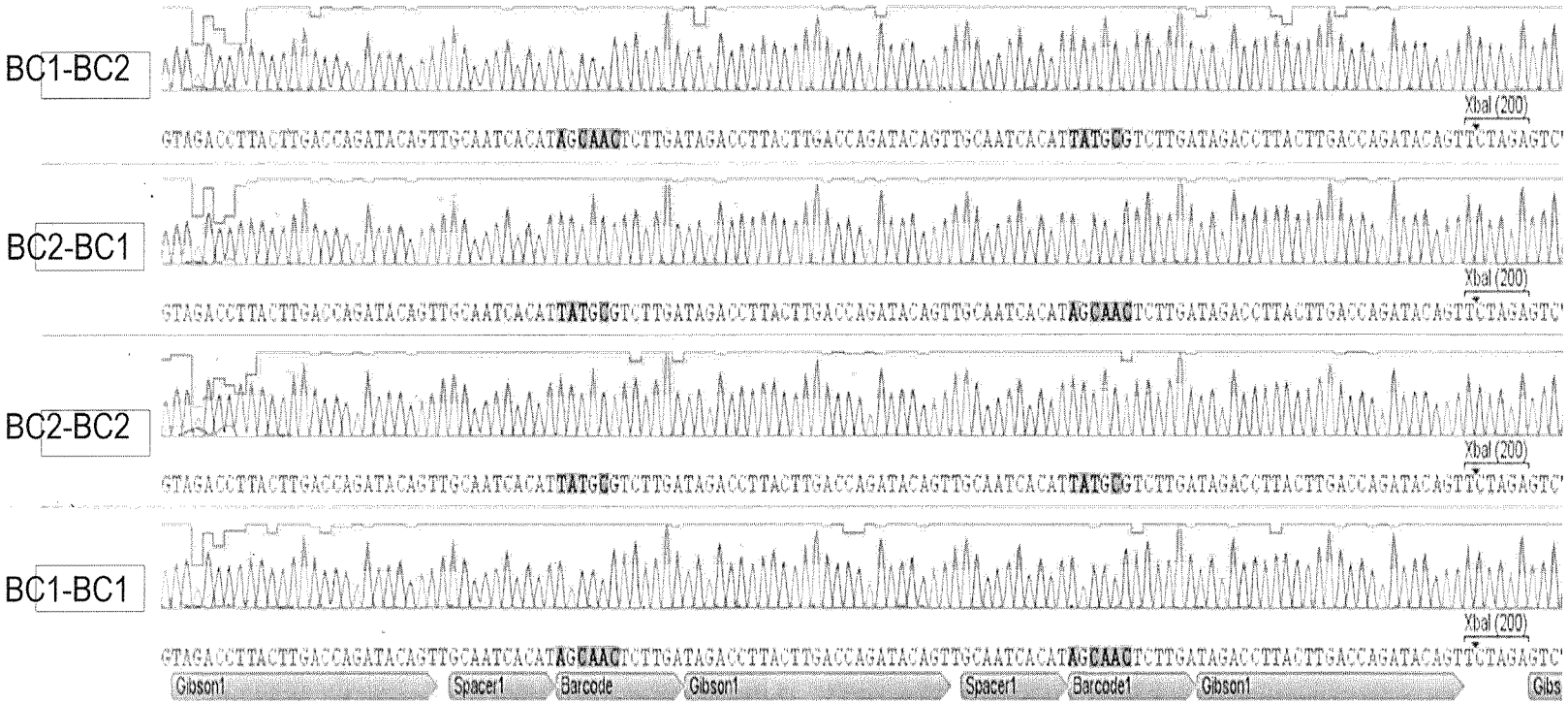
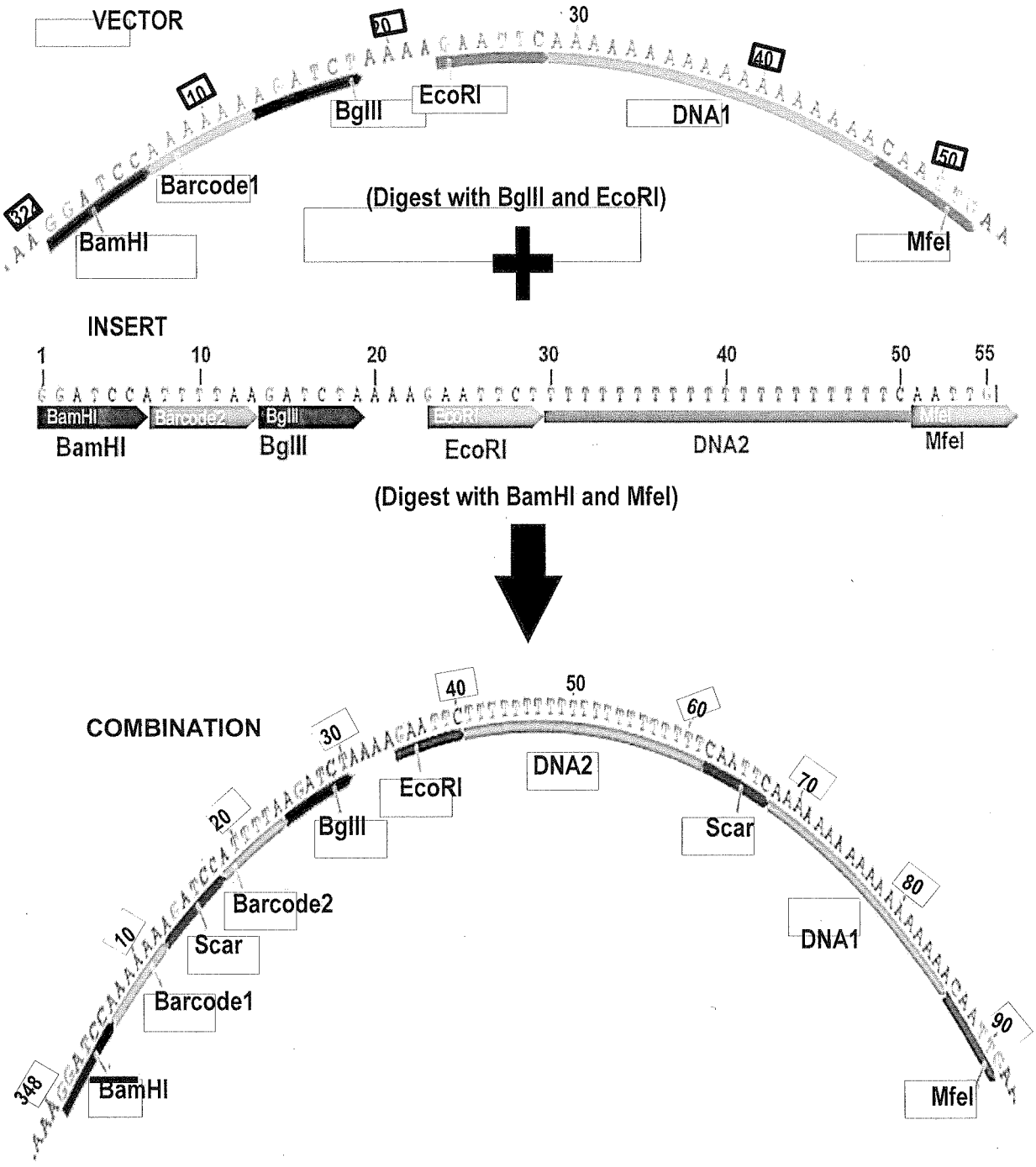



Figure 3

# Figure 4

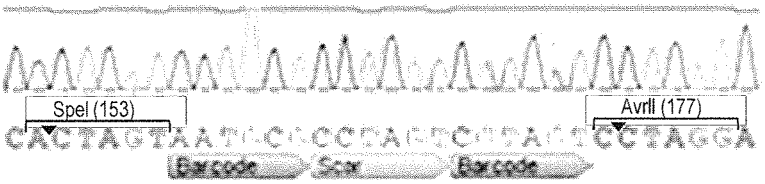

BC1-BC2
BC2-BC1
BC2-BC2
BC1-BC1
XbaI (200)
Gibson1
Spacer1
Barcode
Gibson1
Spacer1
Barcode1
Gibson1
Gibs

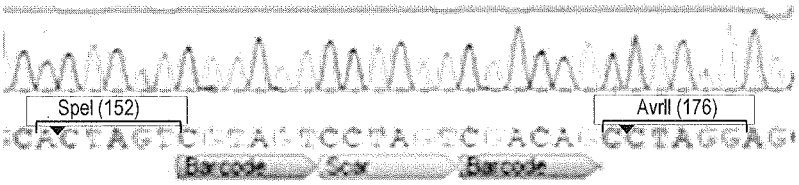
# Figure 5


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

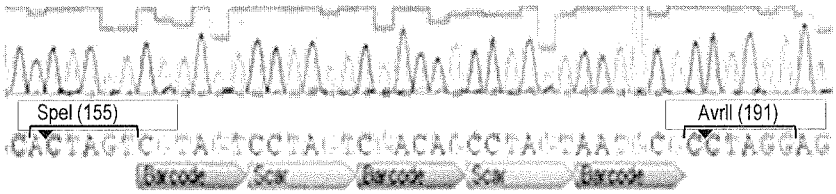
# Figure 6

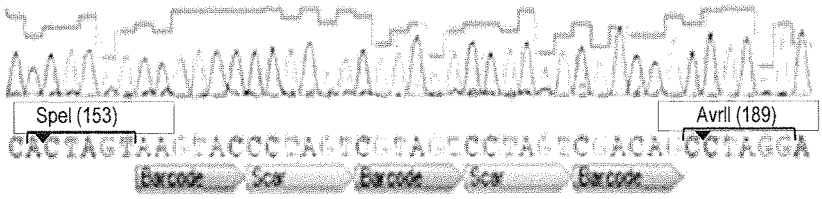
## a) 2x Combination



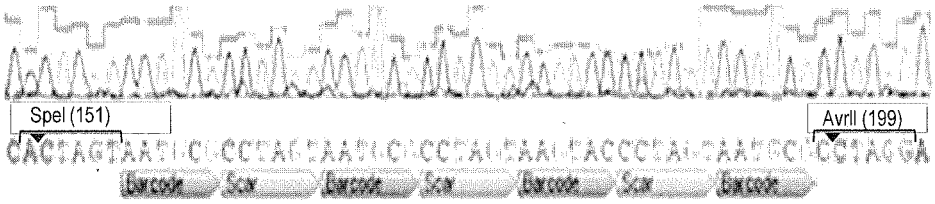



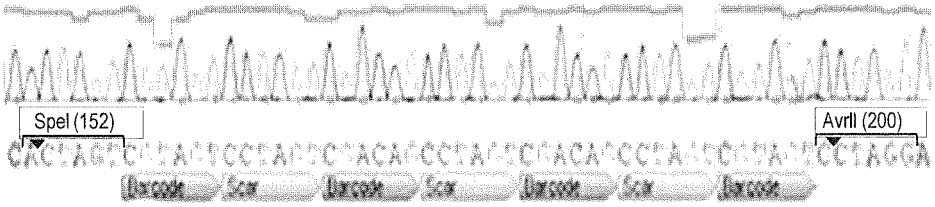

## 3x Combination



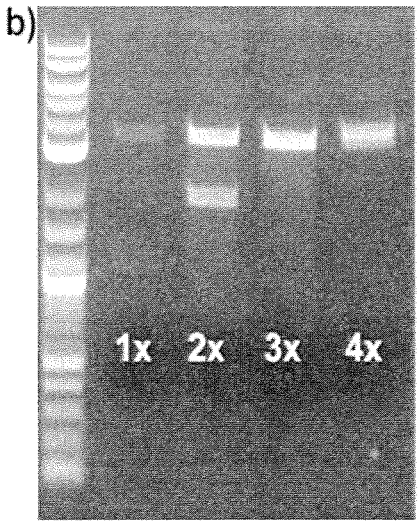




## 4x Combination

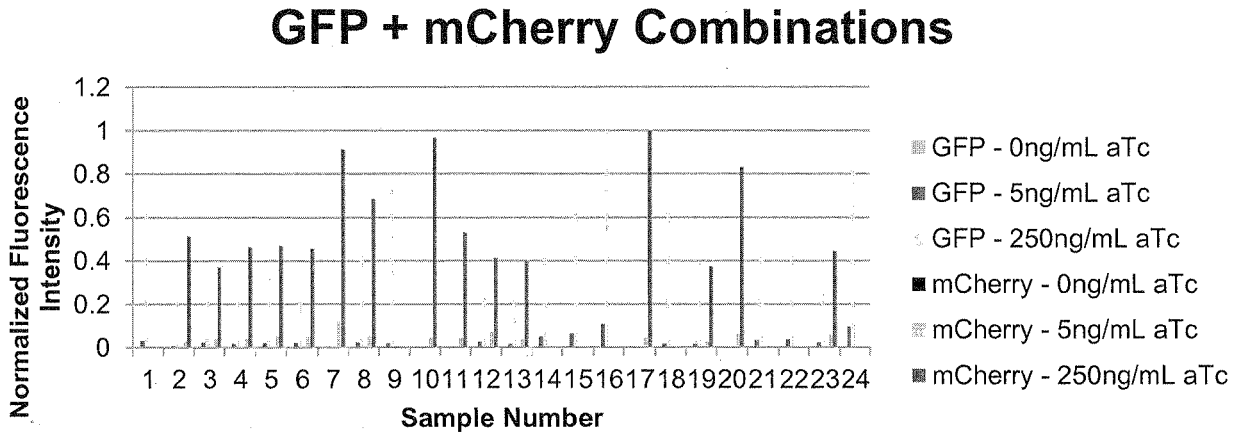


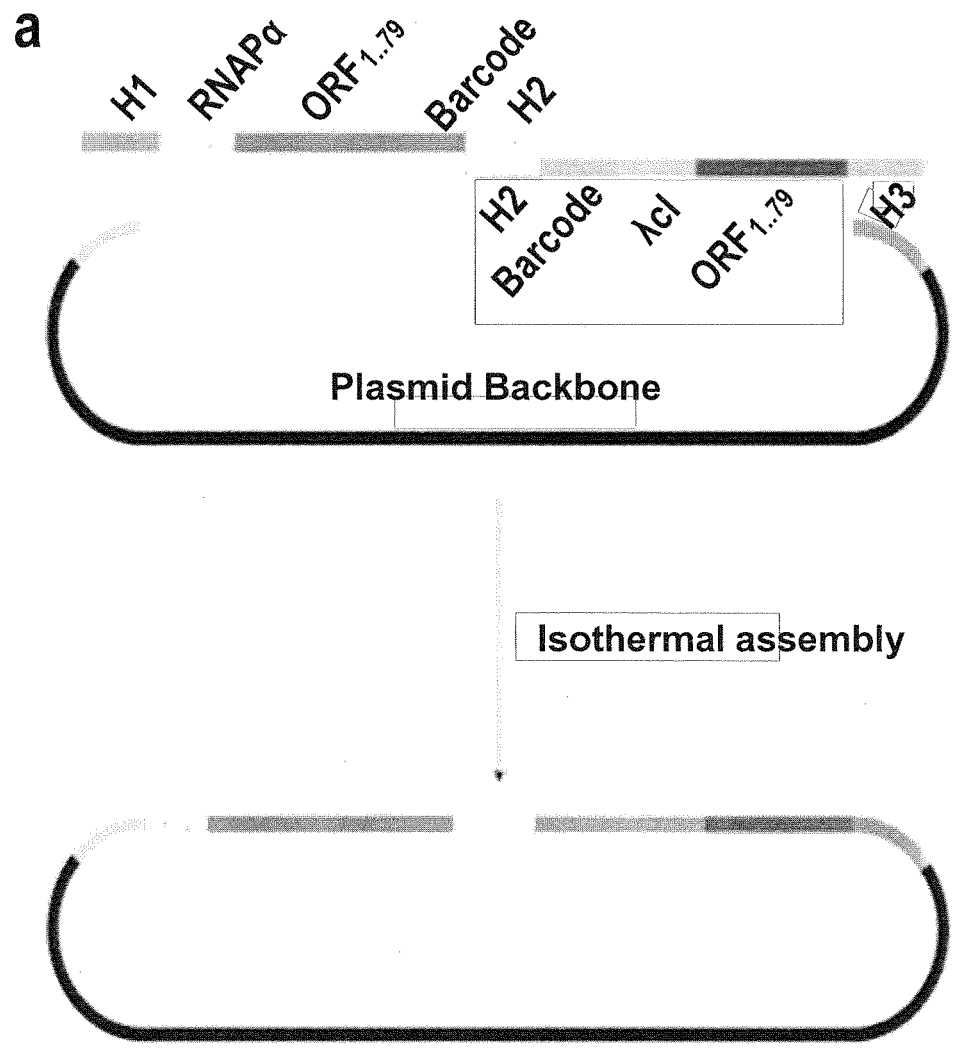
## Figure 6



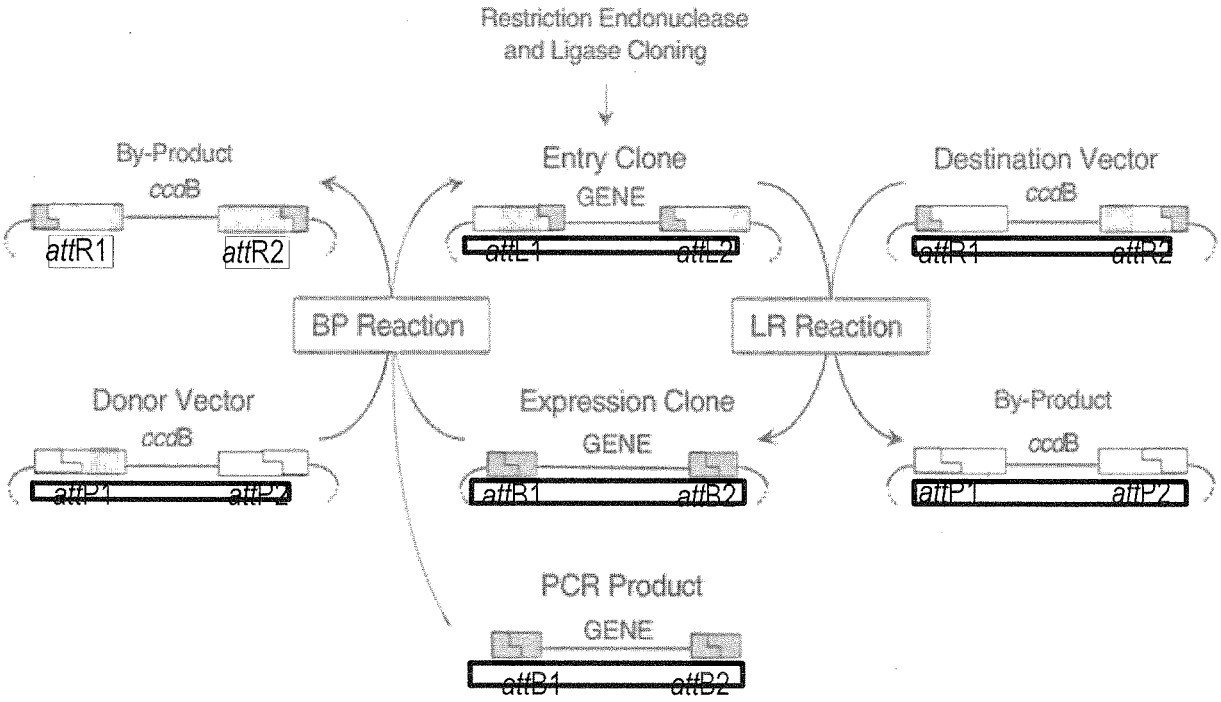

## Figure 7



# Figure 8


a
H1
RNAPα
ORF1..79
Barcode
H2
H2
Barcode
λcI
ORF1..79
H3
Plasmid Backbone
Isothermal assembly

# Figure 9



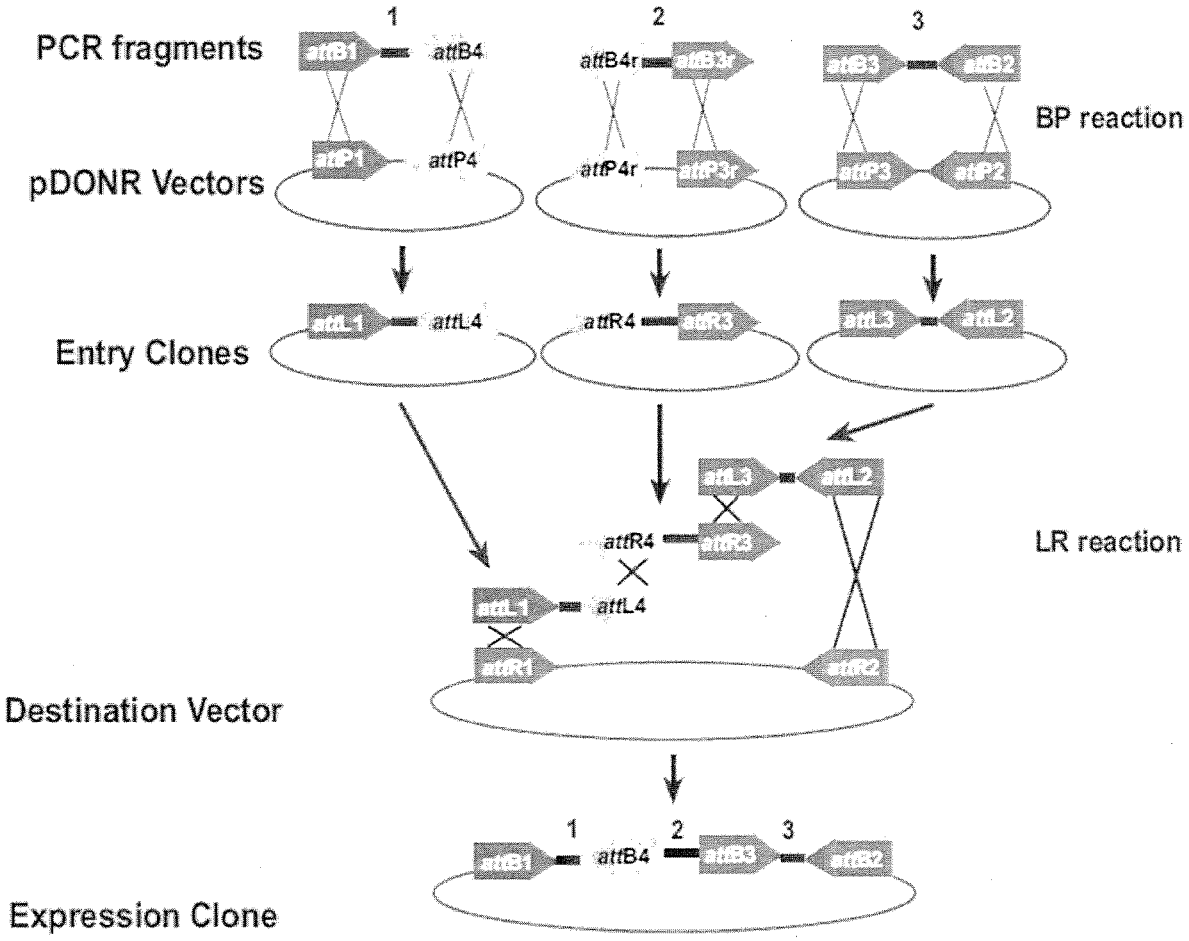

Key:

B1__*__B2 + P1_____P2

L1__*__L2 + R1_____R2

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

# Figure 10




pfgrc.jcvi.org/index.php/gateway_clones/about_knockoutclones.html

**Figure 11**

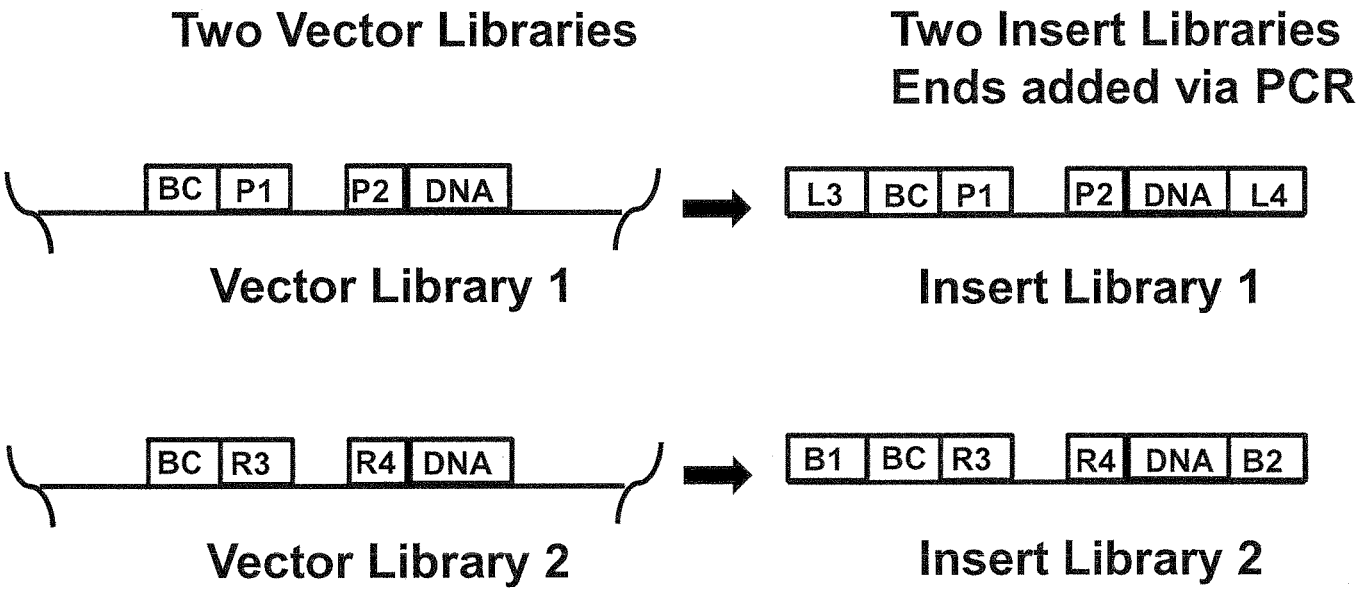

Two Vector Libraries
Two Insert Libraries
Ends added via PCR
BC
P1
P2
DNA
L3
BC
P1
P2
DNA
L4
Vector Library 1
Insert Library 1
BC
R3
R4
DNA
B1
BC
R3
R4
DNA
B2
Vector Library 2
Insert Library 2

**Figure 12**

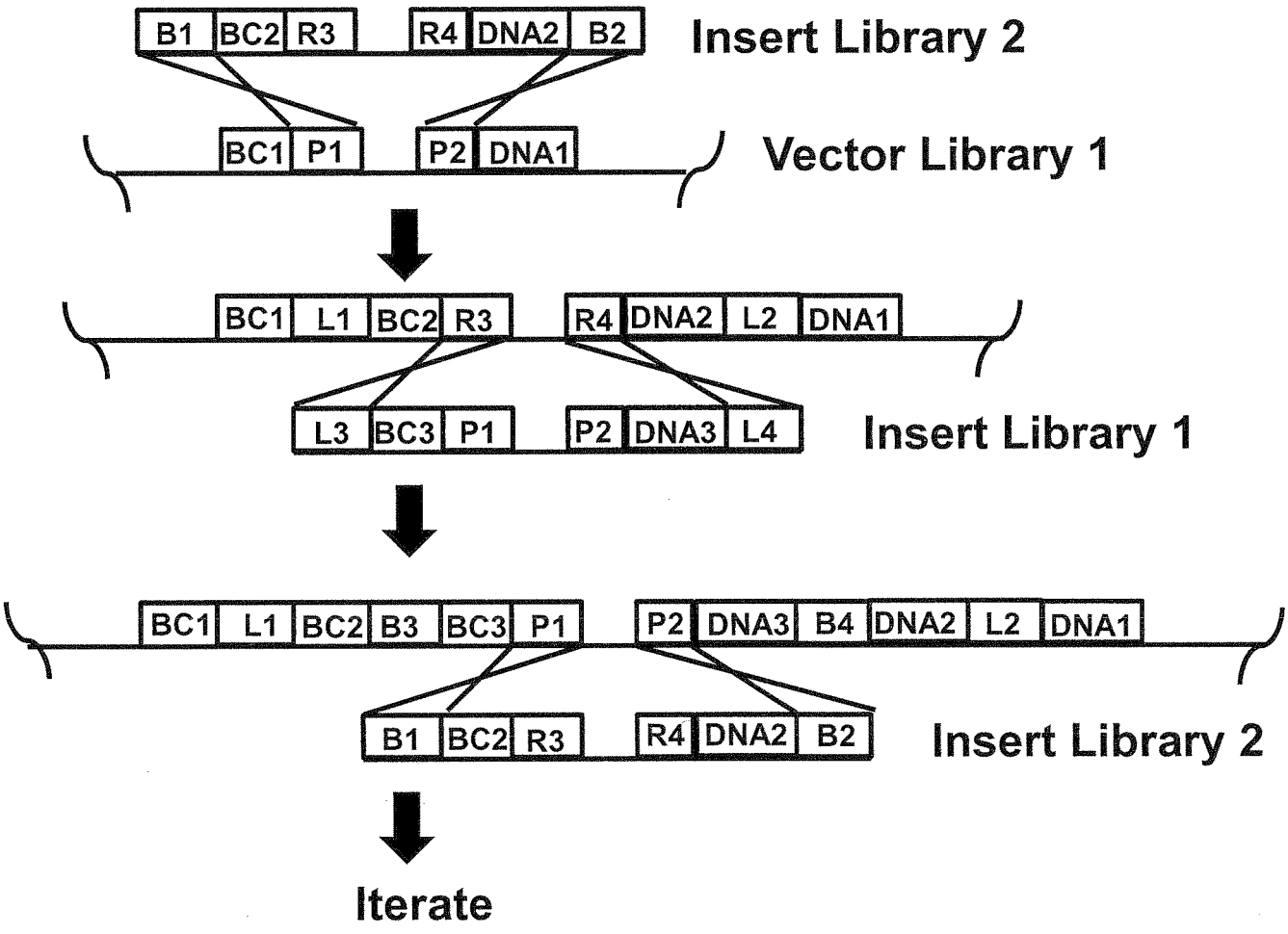

B1
BC2
R3
R4
DNA2
B2
Insert Library 2
BC1
P1
P2
DNA1
Vector Library 1
BC1
L1
BC2
R3
R4
DNA2
L2
DNA1
L3
BC3
P1
P2
DNA3
L4
Insert Library 1
BC1
L1
BC2
B3
BC3
P1
P2
DNA3
B4
DNA2
L2
DNA1
B1
BC2
R3
R4
DNA2
B2
Insert Library 2
Iterate

**Figure 13**

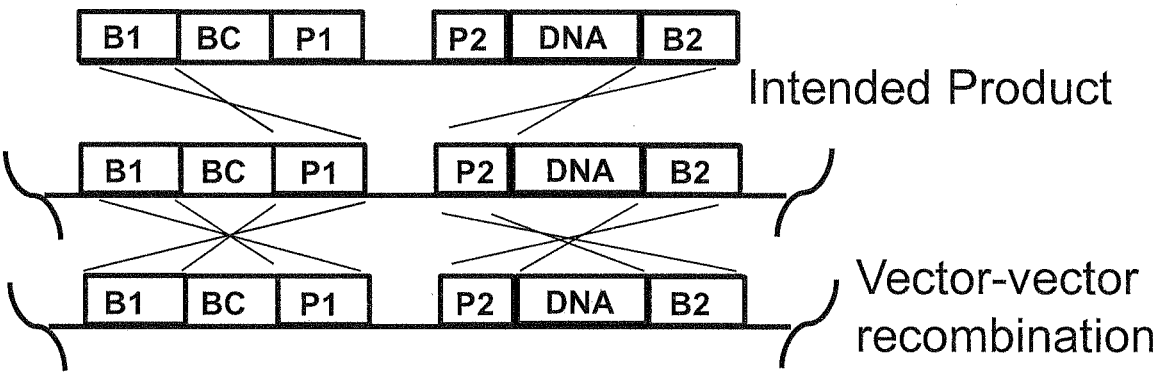
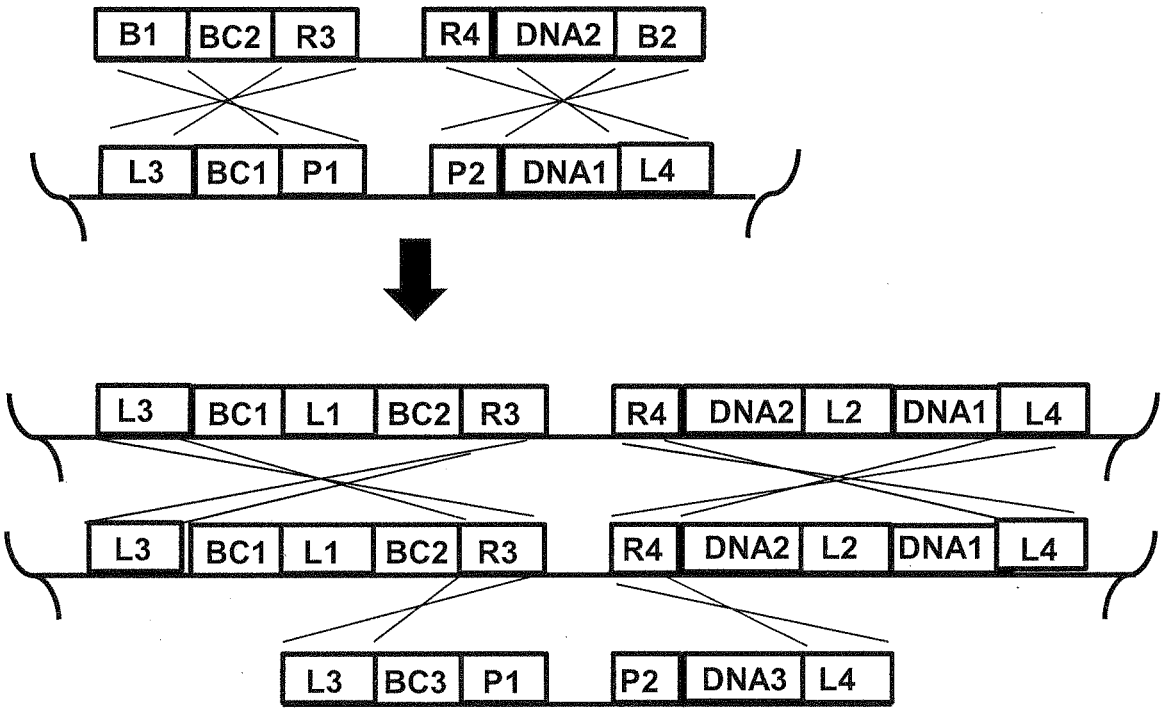

B1
BC
P1
P2
DNA
B2
Intended Product
B1
BC
P1
P2
DNA
B2
B1
BC
P1
P2
DNA
B2
Vector-vector
recombination

**Figure 14**


B1
BC2
R3
R4
DNA2
B2
L3
BC1
P1
P2
DNA1
L4
L3
BC1
L1
BC2
R3
R4
DNA2
L2
DNA1
L4
L3
BC1
L1
BC2
R3
R4
DNA2
L2
DNA1
L4
L3
BC3
P1
P2
DNA3
L4

# Figure 15

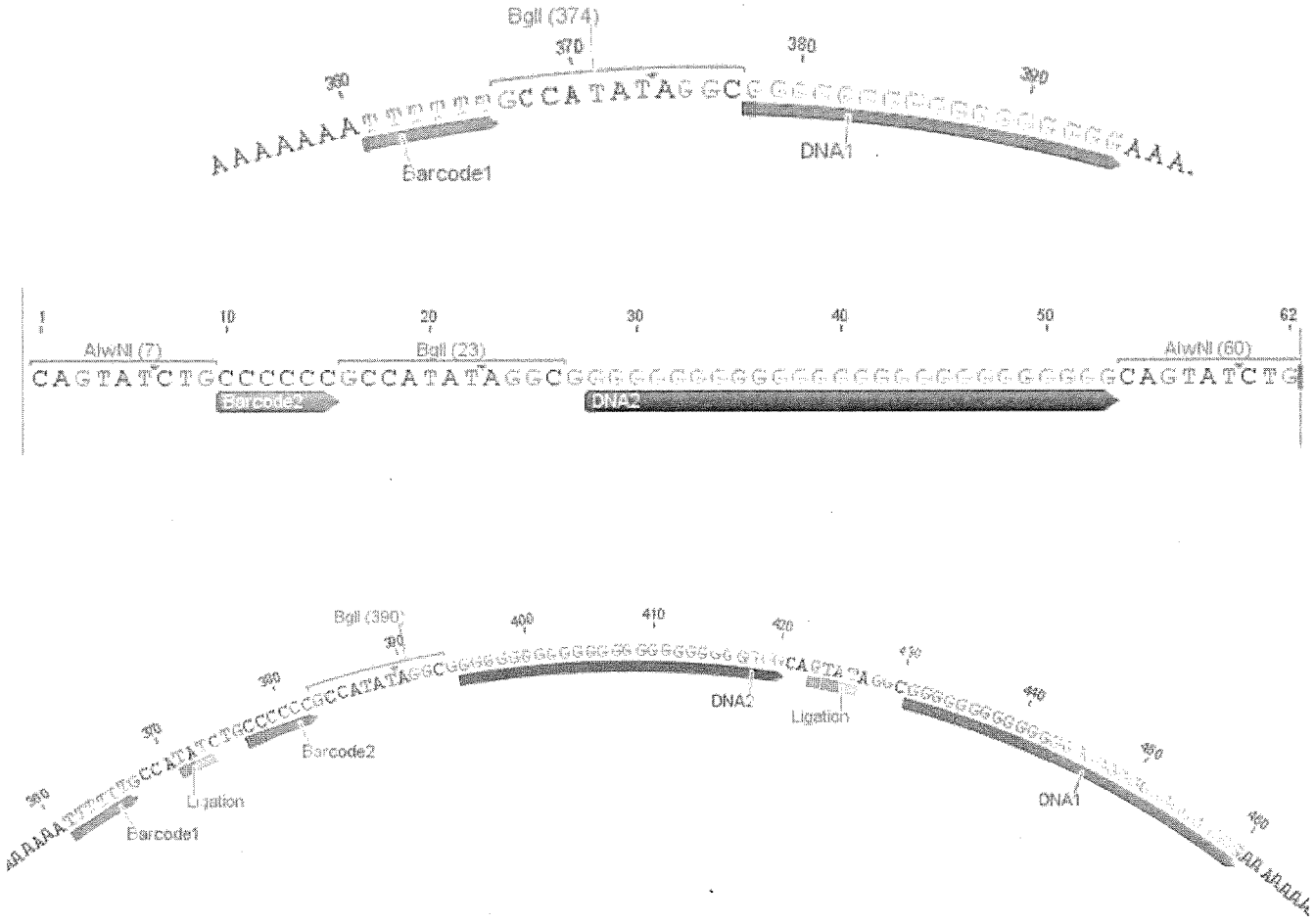
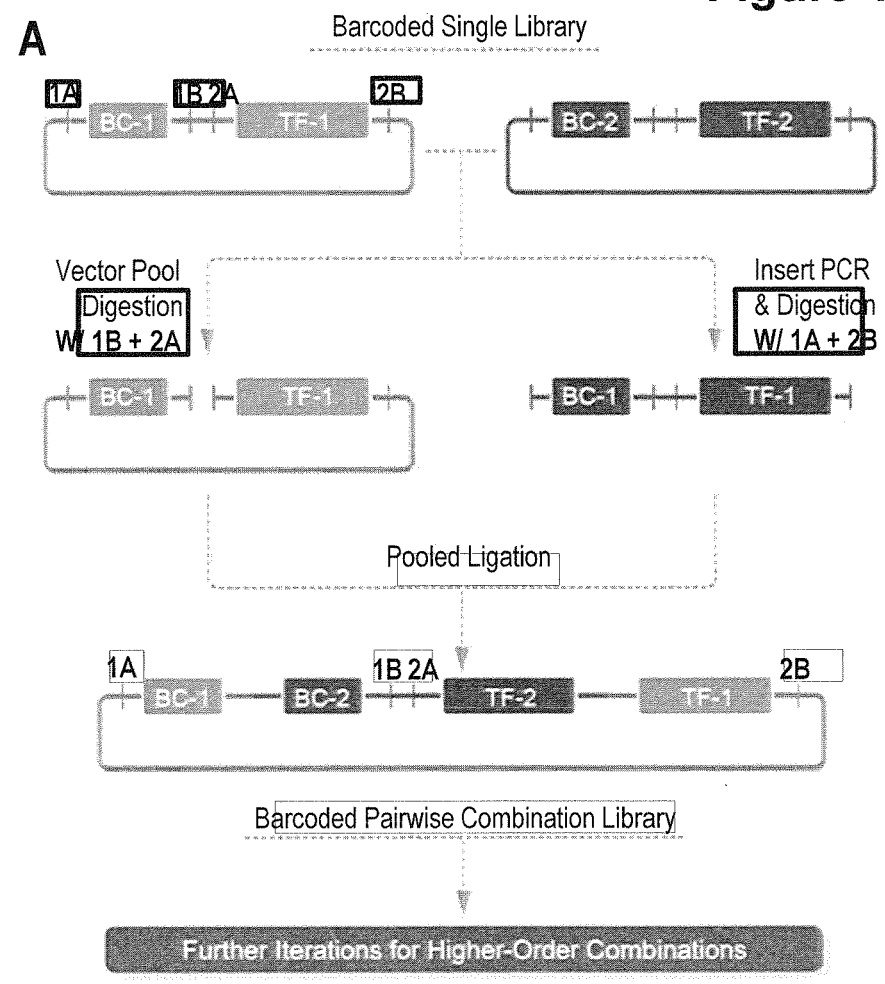

BglI (374)
370
380
390
AAAAAAA
GCCATATAGGCG
Barcode1
DNA1
AAA
1
10
20
30
40
50
62
AlwNI (7)
BglI (23)
AlwNI (60)
CAGTATCTGCCCCCCGCCATATAGGCG
CAGTATCTG
Barcode2
DNA2
BglI (390)
400
410
420
430
440
450
460
CAGTA
DNA2
Ligation
Barcode2
Ligation
Barcode1
DNA1

**Figure 16**


A
Barcoded Single Library
1A
1B 2A
2B
BC-1
TF-1
BC-2
TF-2
Vector Pool
Digestion
W/ 1B + 2A
Insert PCR
& Digestion
W/ 1A + 2B
Pooled Ligation
Barcoded Pairwise Combination Library
Further Iterations for Higher-Order Combinations

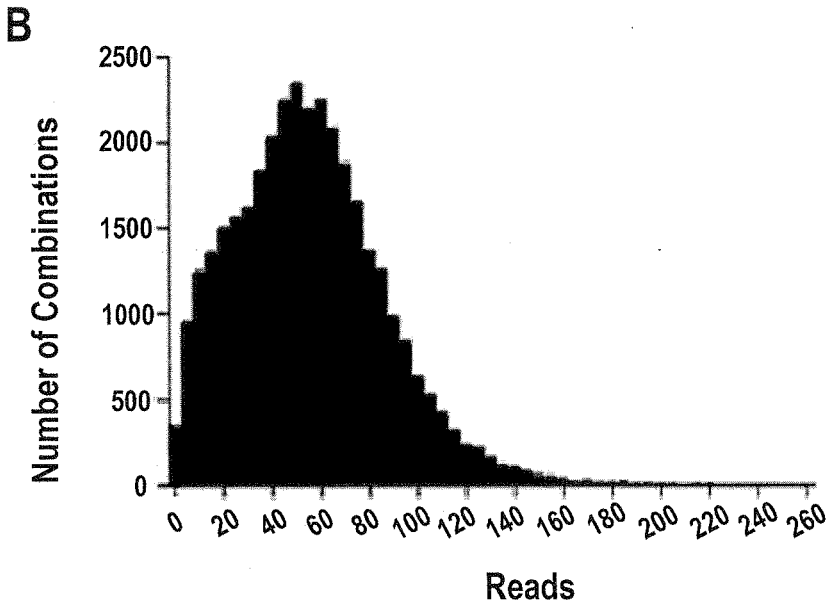

B
Number of Combinations
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
140
160
180
200
220
240
260
Reads

**Figure 16**

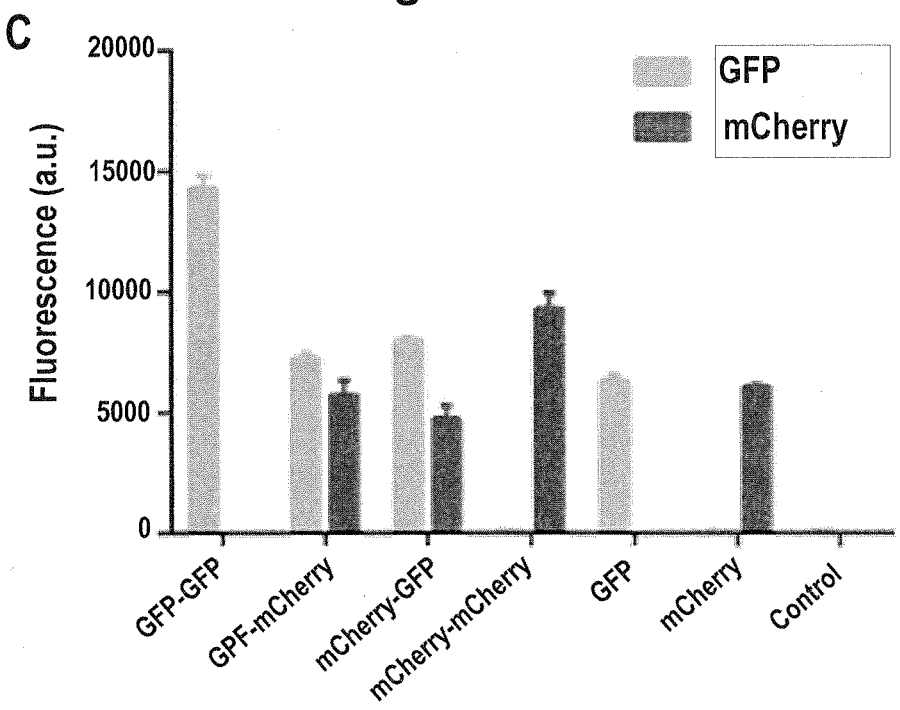



**Figure 17**

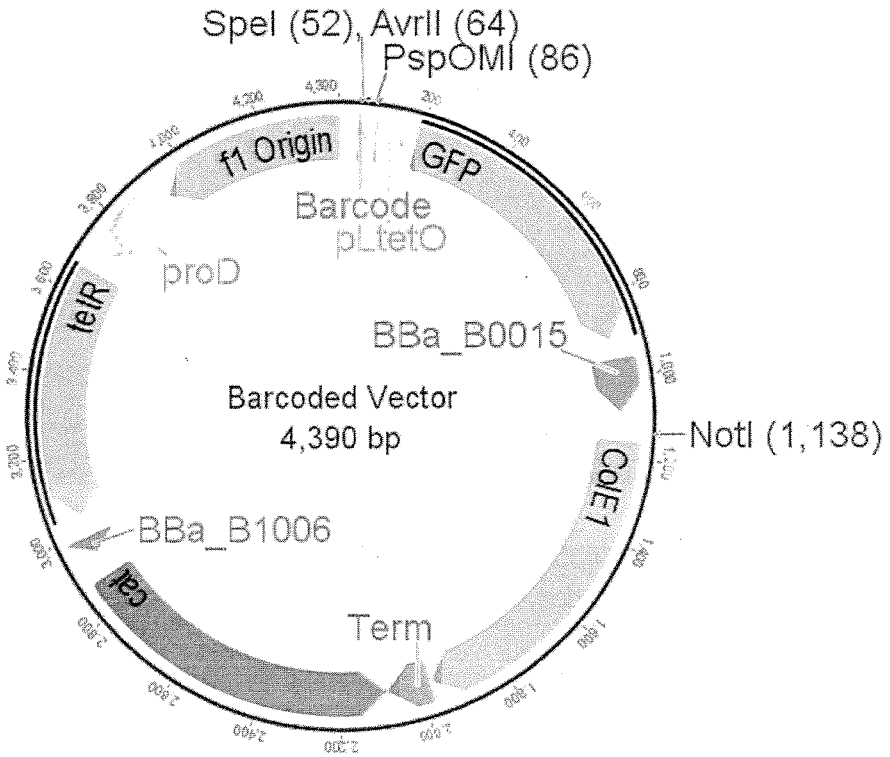
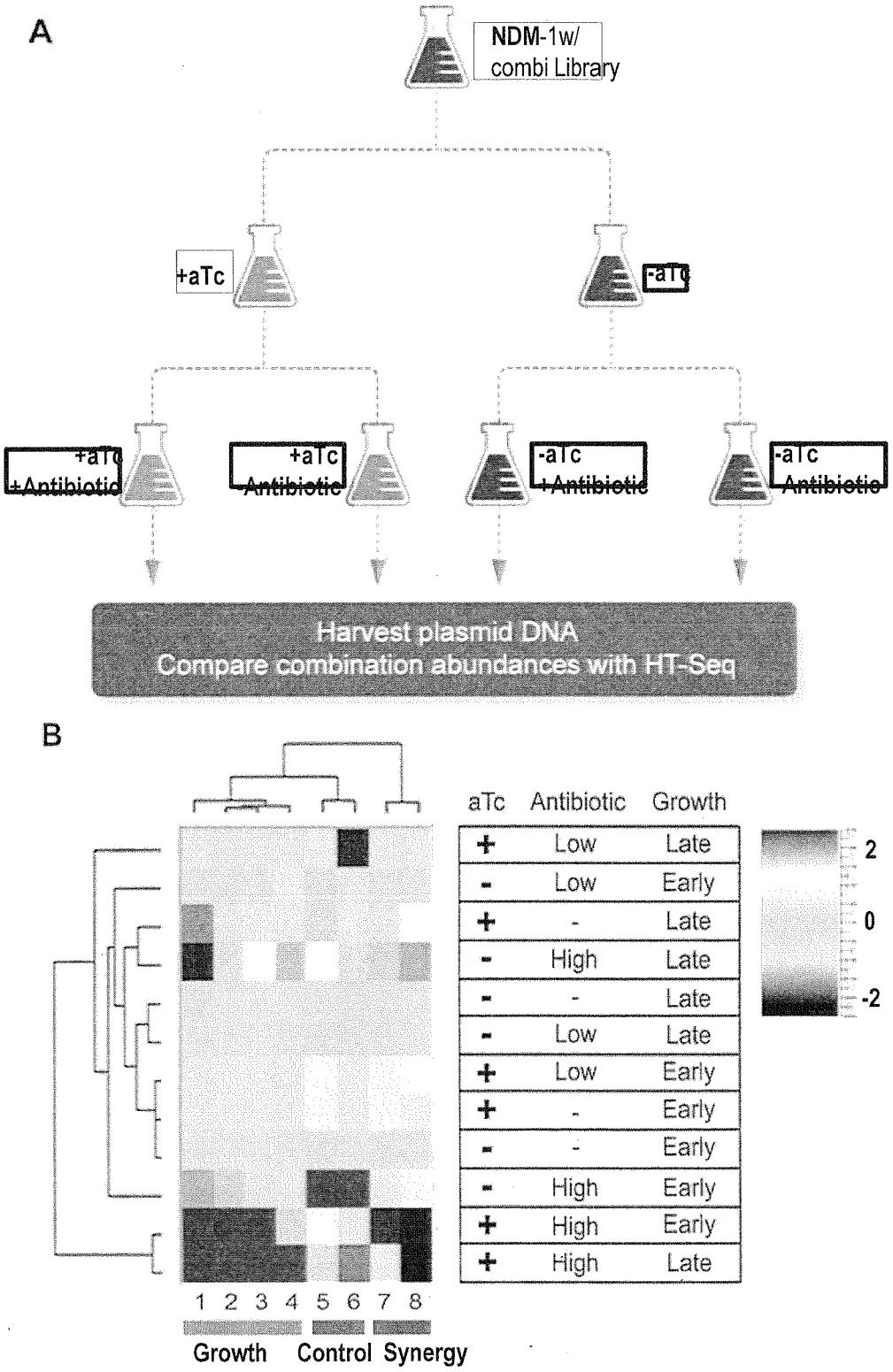


**Figure 18**


A
NDM-1w/
combi Library
+aTc
-aTc
+aTc
+Antibiotic
+aTc
Antibiotic
-aTc
+Antibiotic
-aTc
Antibiotic
Harvest plasmid DNA
Compare combination abundances with HT-Seq
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

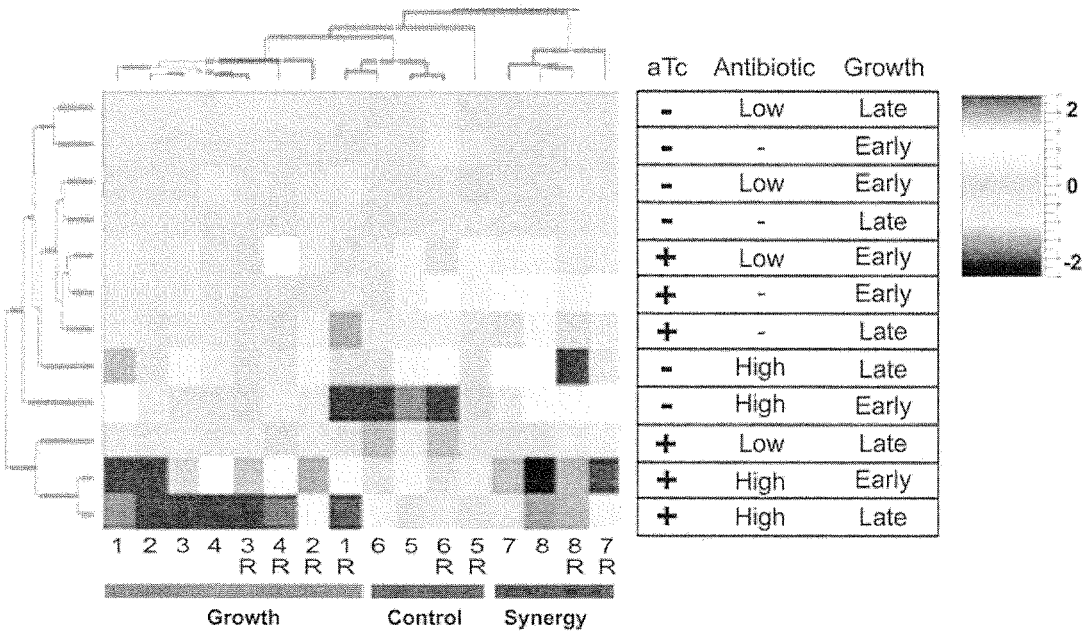
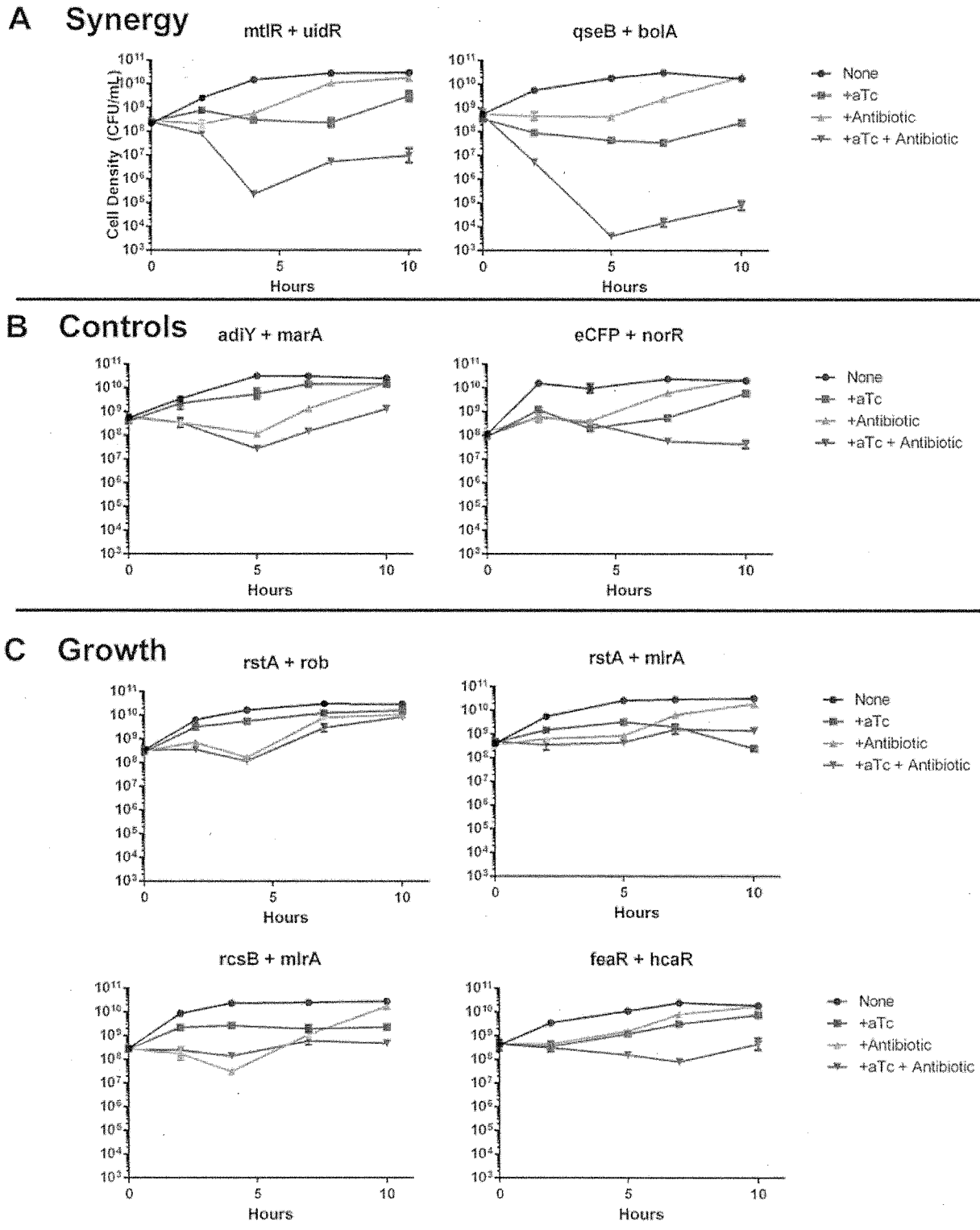

aTc
Antibiotic
Growth
- Low Late
- - Early
- Low Early
- - Late
+ Low Early
+ - Early
+ - Late
- High Late
- High Early
+ Low Late
+ High Early
+ High Late
2
0
-2
1 2 3 4 3R 4R 2R 1R 6 5 6R 5R 7 8 8R 7R
Growth
Control
Synergy

**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

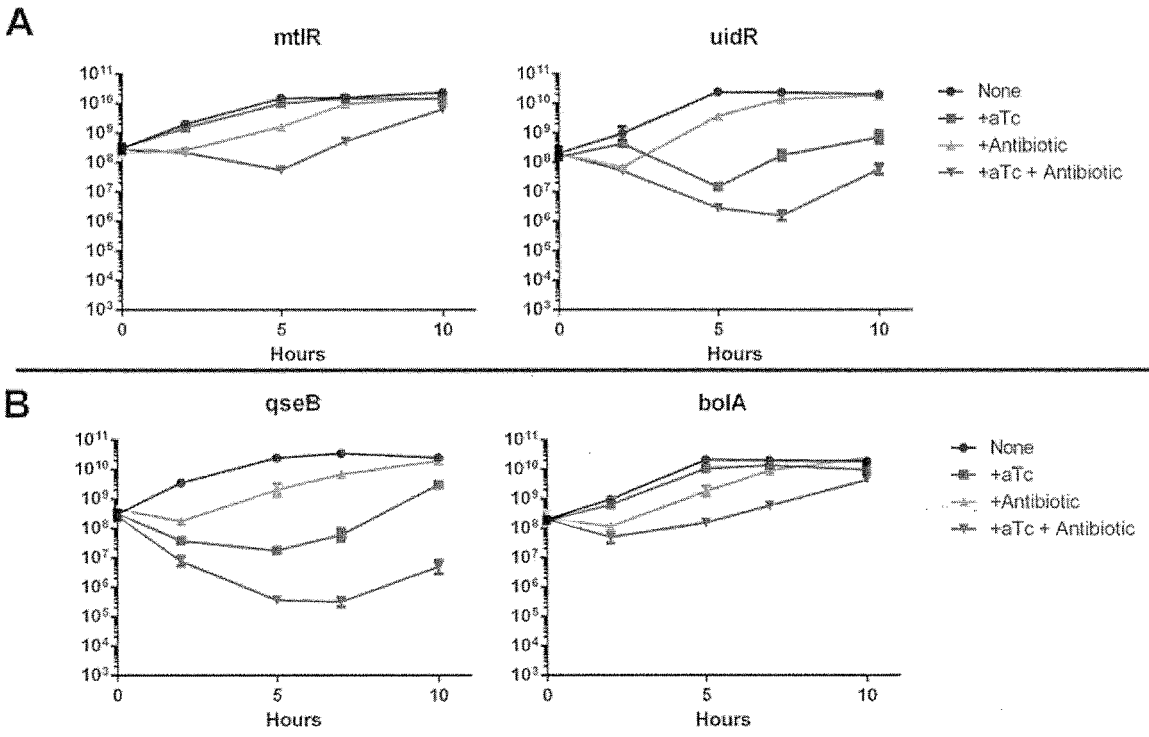
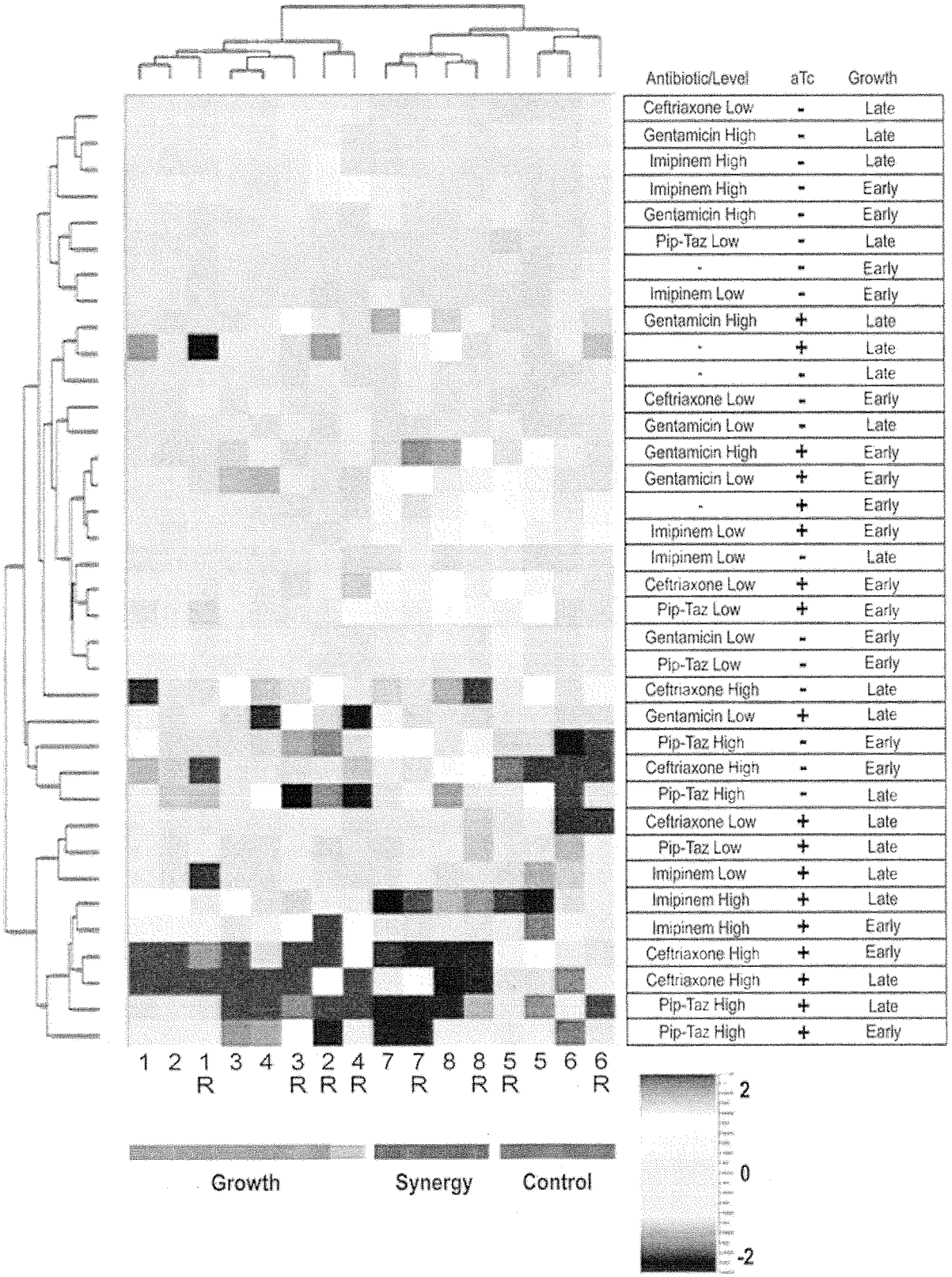

A
mtlR
uidR
Hours
Hours
None
+aTc
+Antibiotic
+aTc + Antibiotic
B
qseB
bolA
Hours
Hours
None
+aTc
+Antibiotic
+aTc + Antibiotic

**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

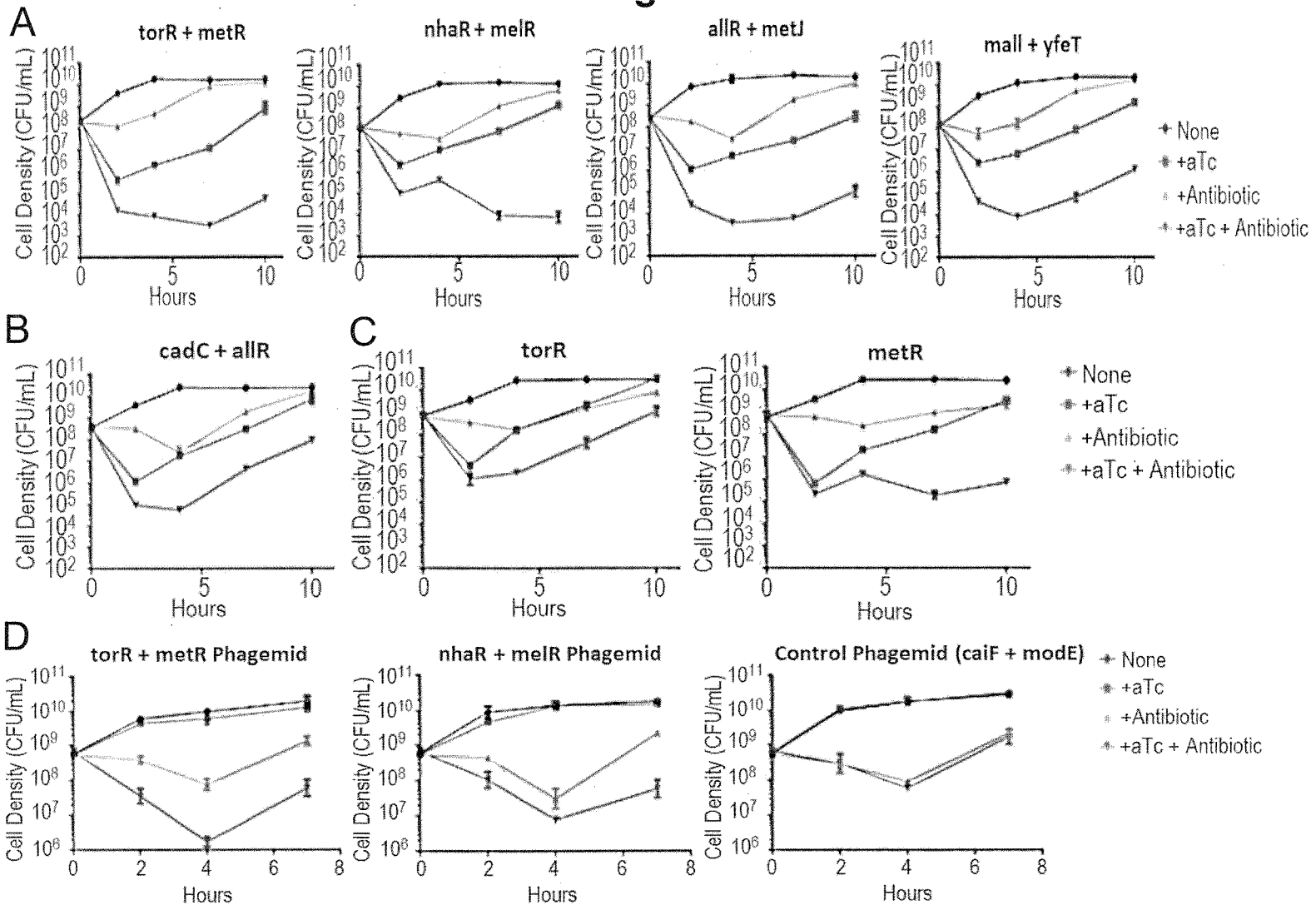

A
torR + metR
nhaR + melR
allR + metJ
mall + yfeT
B
cadC + allR
C
torR
metR
D
torR + metR Phagemid
nhaR + melR Phagemid
Control Phagemid (caiF + modE)
Cell Density (CFU/mL)
Hours
None
+aTc
+Antibiotic
+aTc + Antibiotic

**Figure 24**

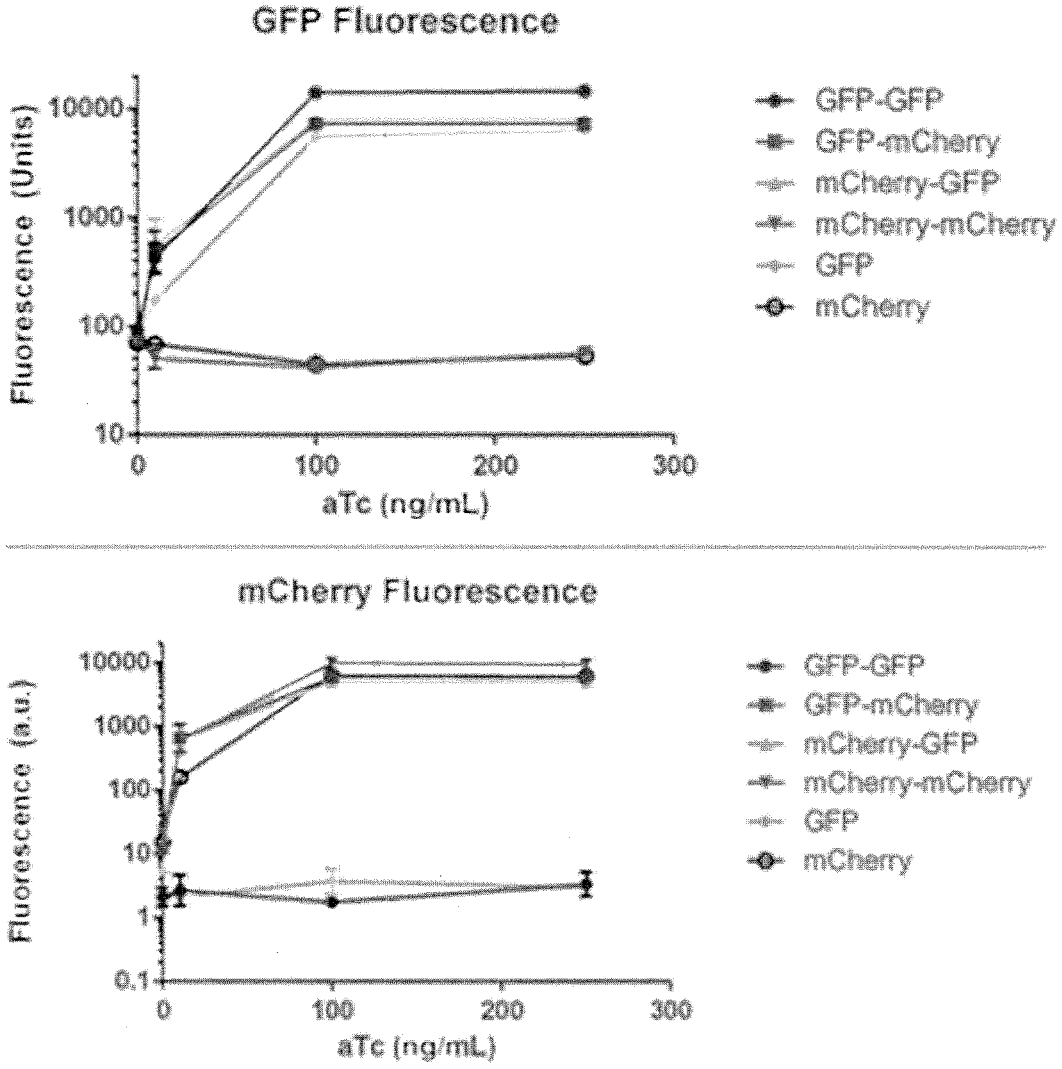

GFP Fluorescence
Fluorescence (Units)
10000
1000
100
10
0
100
200
300
aTc (ng/mL)
GFP-GFP
GFP-mCherry
mCherry-GFP
mCherry-mCherry
GFP
mCherry
mCherry Fluorescence
Fluorescence (a.u.)
10000
1000
100
10
1
0.1
0
100
200
300
aTc (ng/mL)
GFP-GFP
GFP-mCherry
mCherry-GFP
mCherry-mCherry
GFP
mCherry

Figure 25

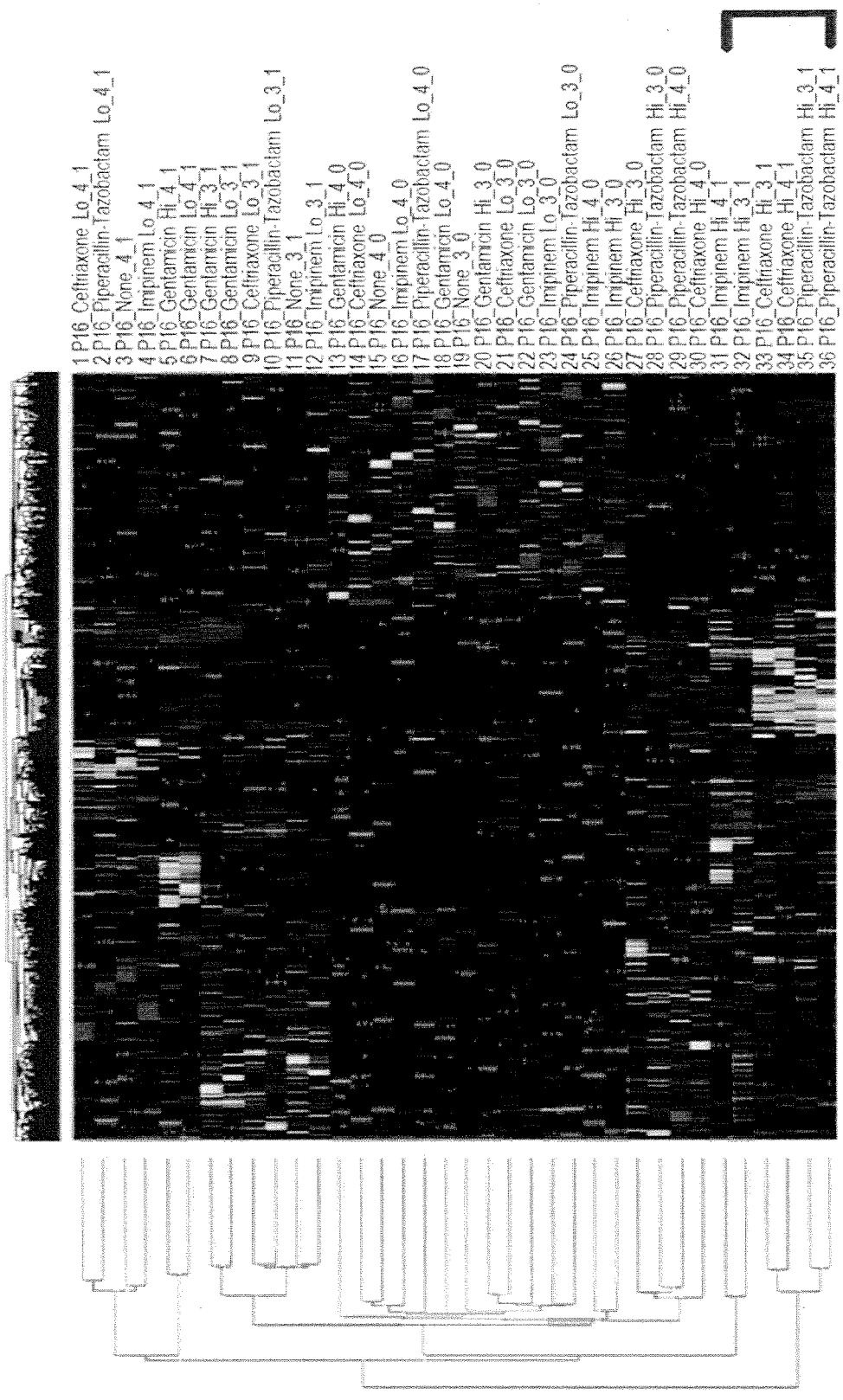

1 P16_Ceftriaxone Lo_4_1
2 P16_Piperacillin-Tazobactam Lo_4_1
3 P16_None_4_1
4 P16_Imipinem Lo_4_1
5 P16_Gentamicin Hi_4_1
6 P16_Gentamicin Lo_4_1
7 P16_Gentamicin Hi_3_1
8 P16_Gentamicin Lo_3_1
9 P16_Ceftriaxone Lo_3_1
10 P16_Piperacillin-Tazobactam Lo_3_1
11 P16_None_3_1
12 P16_Imipinem Lo_3_1
13 P16_Gentamicin Hi_4_0
14 P16_Ceftriaxone Lo_4_0
15 P16_None_4_0
16 P16_Imipinem Lo_4_0
17 P16_Piperacillin-Tazobactam Lo_4_0
18 P16_Gentamicin Lo_4_0
19 P16_None_3_0
20 P16_Gentamicin Hi_3_0
21 P16_Ceftriaxone Lo_3_0
22 P16_Gentamicin Lo_3_0
23 P16_Imipinem Lo_3_0
24 P16_Piperacillin-Tazobactam Lo_3_0
25 P16_Imipinem Hi_4_0
26 P16_Imipinem Hi_3_0
27 P16_Ceftriaxone Hi_3_0
28 P16_Piperacillin-Tazobactam Hi_3_0
29 P16_Piperacillin-Tazobactam Hi_4_0
30 P16_Ceftriaxone Hi_4_0
31 P16_Imipinem Hi_4_1
32 P16_Imipinem Hi_3_1
33 P16_Ceftriaxone Hi_3_1
34 P16_Ceftriaxone Hi_4_1
35 P16_Piperacillin-Tazobactam Hi_3_1
36 P16_Piperacillin-Tazobactam Hi_4_1

**Figure 26**

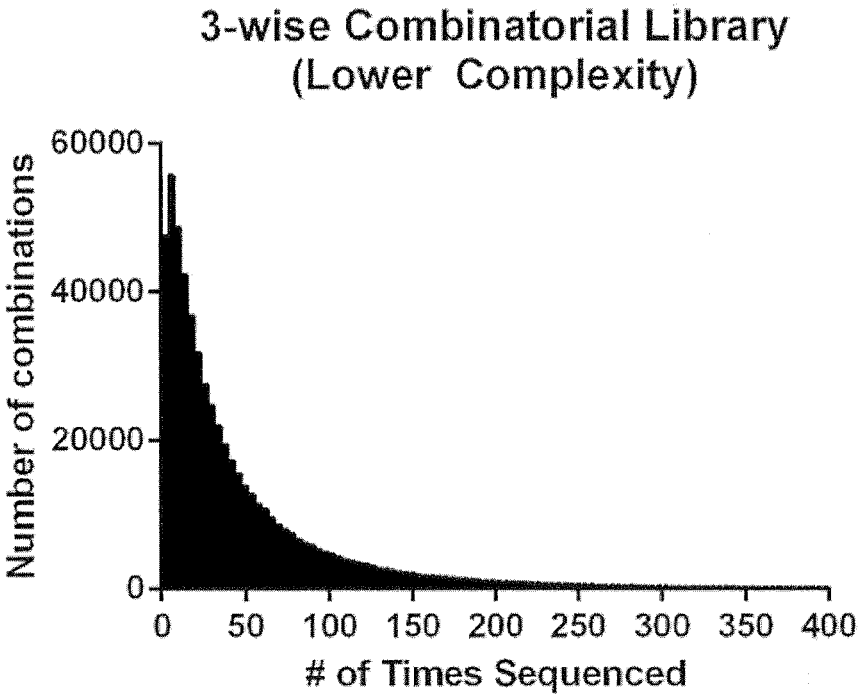
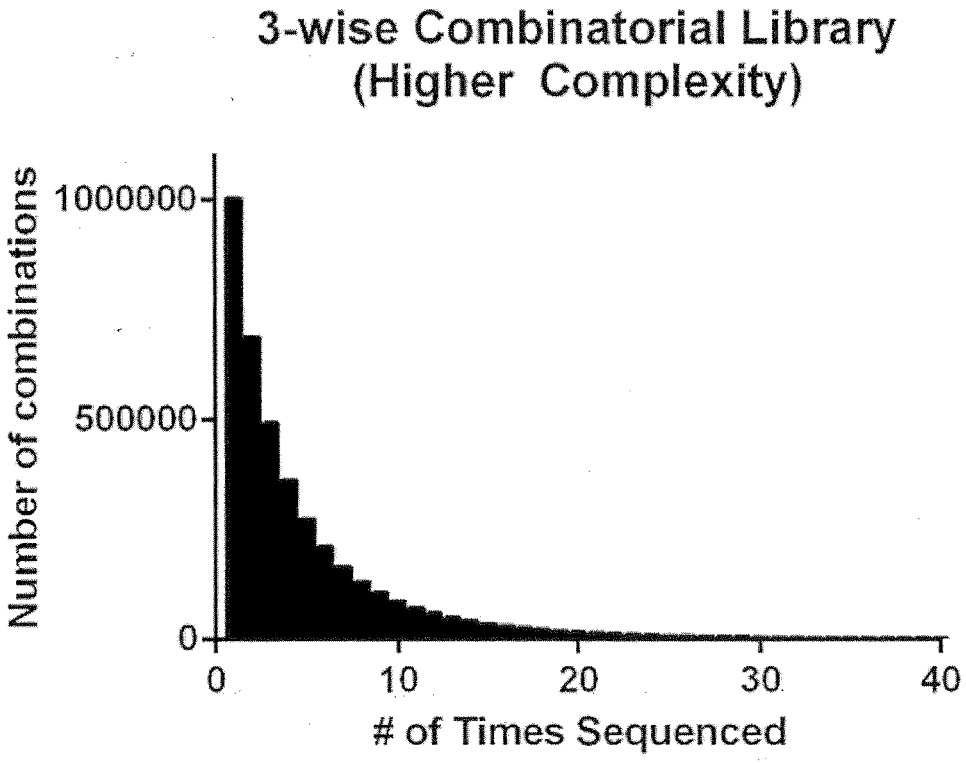
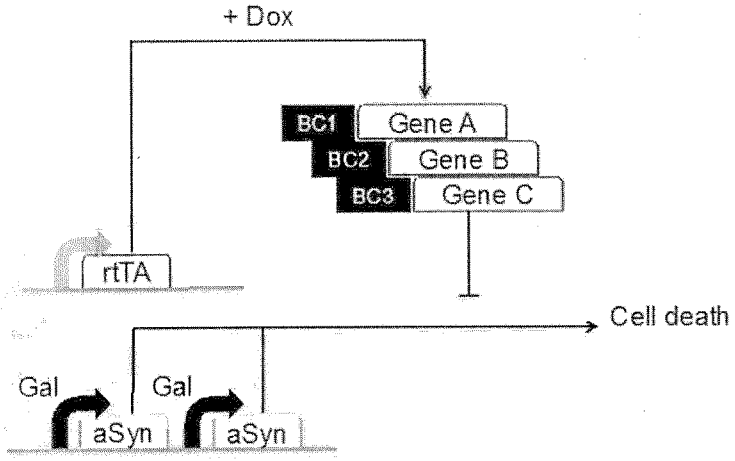



**Figure 27**



# Figure 28


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

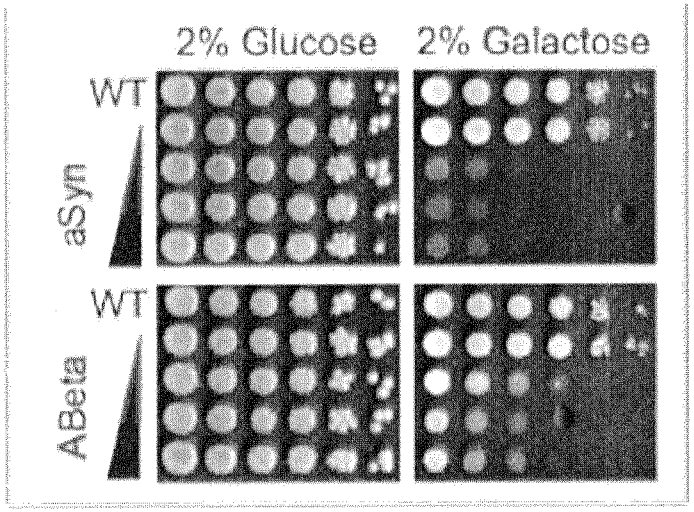

2% Glucose
2% Galactose
WT
aSyn
WT
ABeta

**Figure 29**

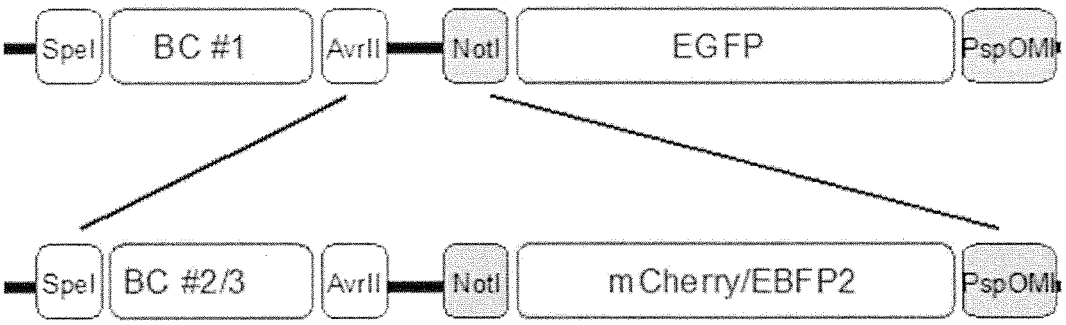



**Figure 30**

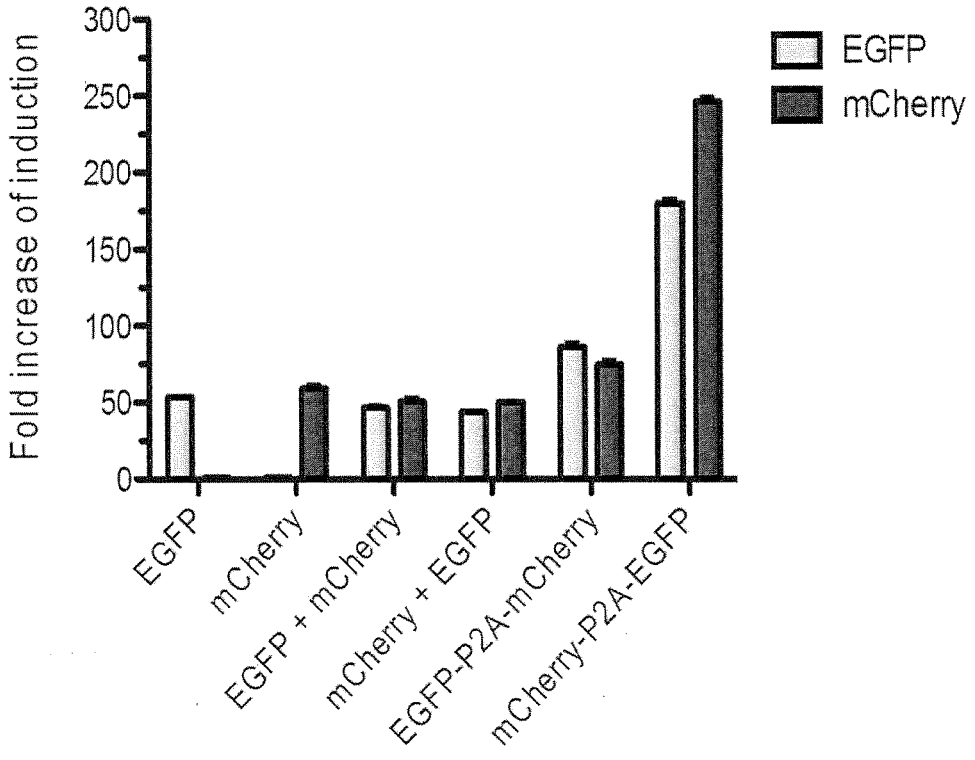


**Figure 31**

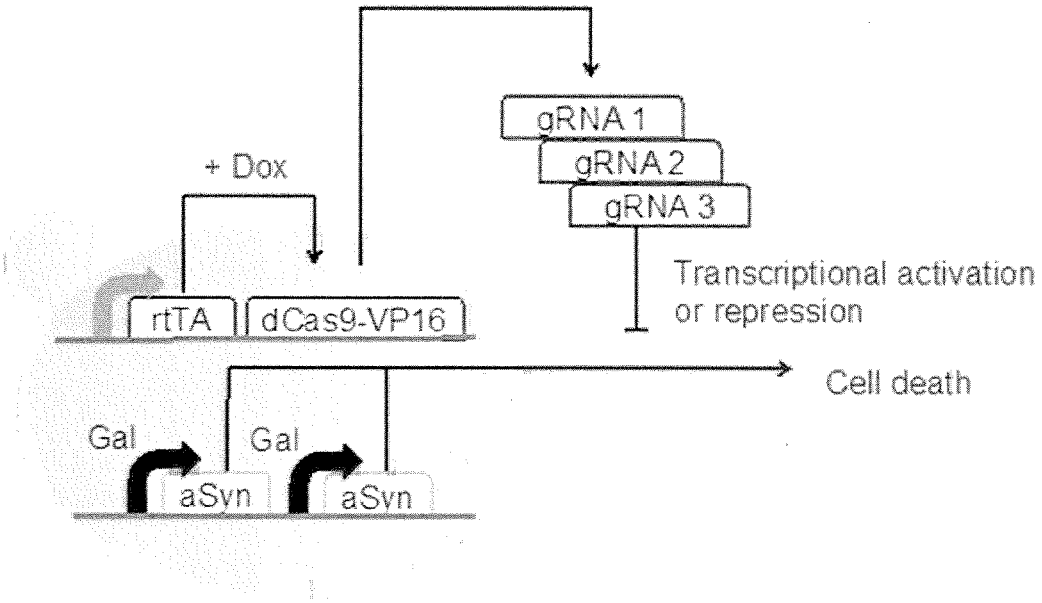



**Figure 32**

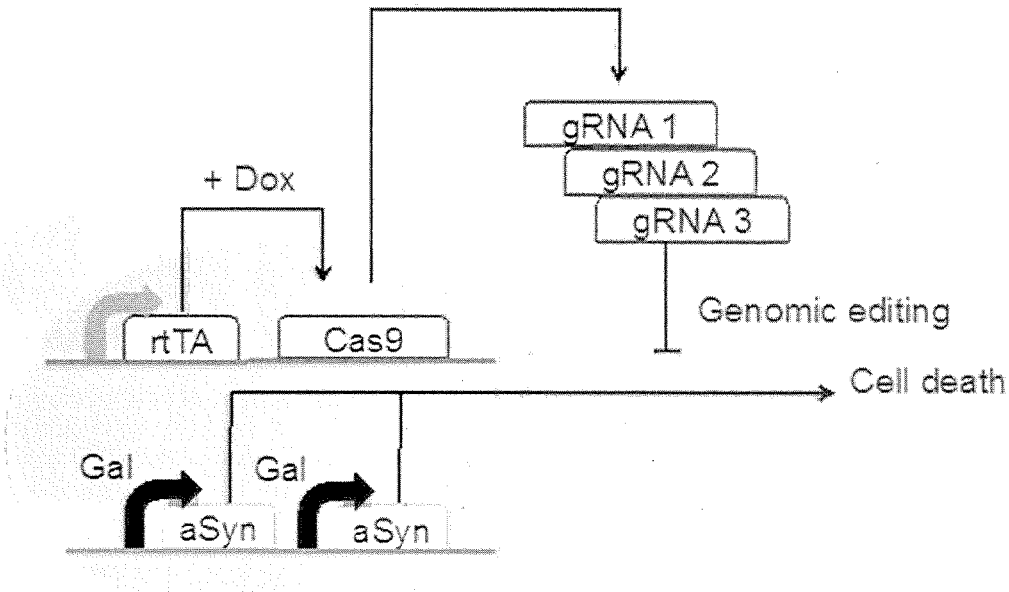
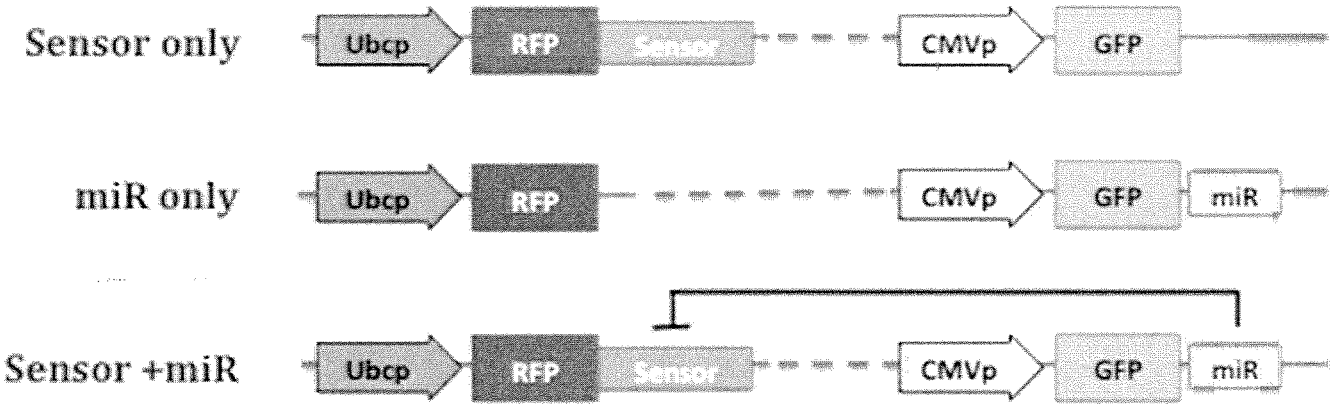
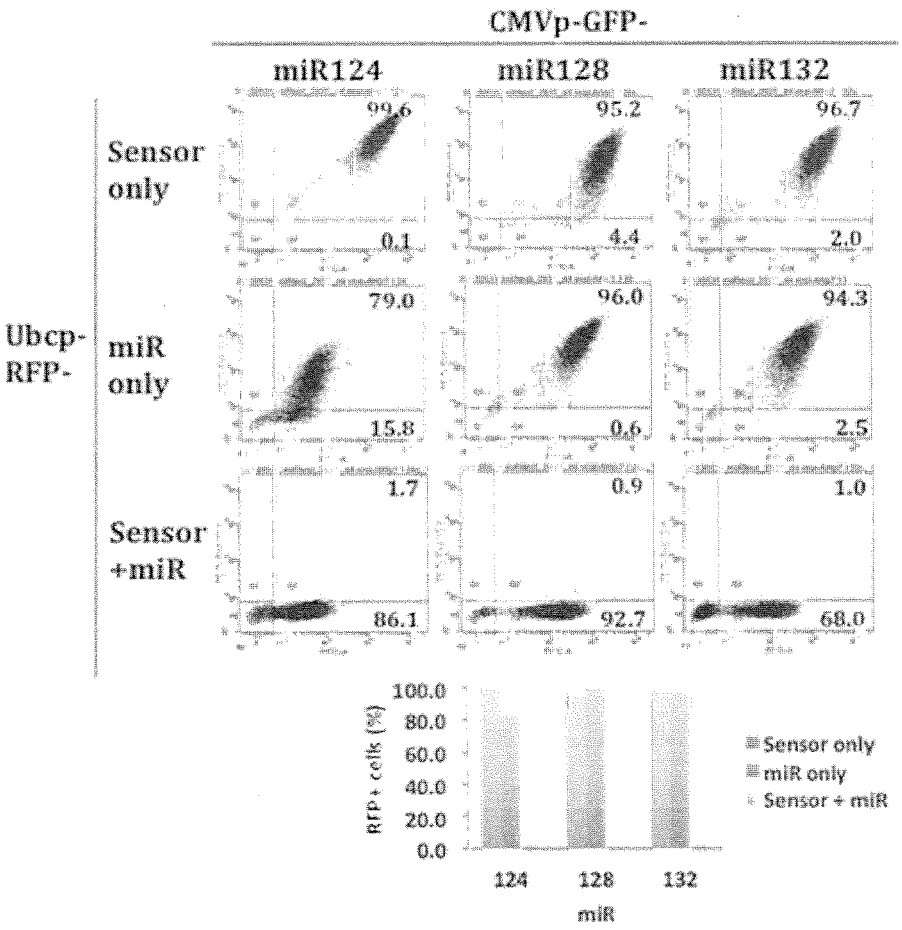
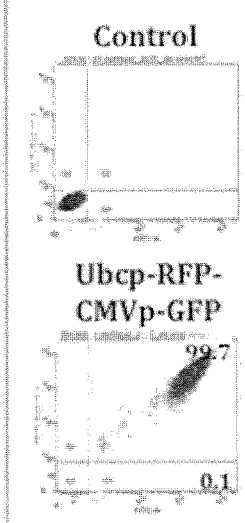


**Figure 33**

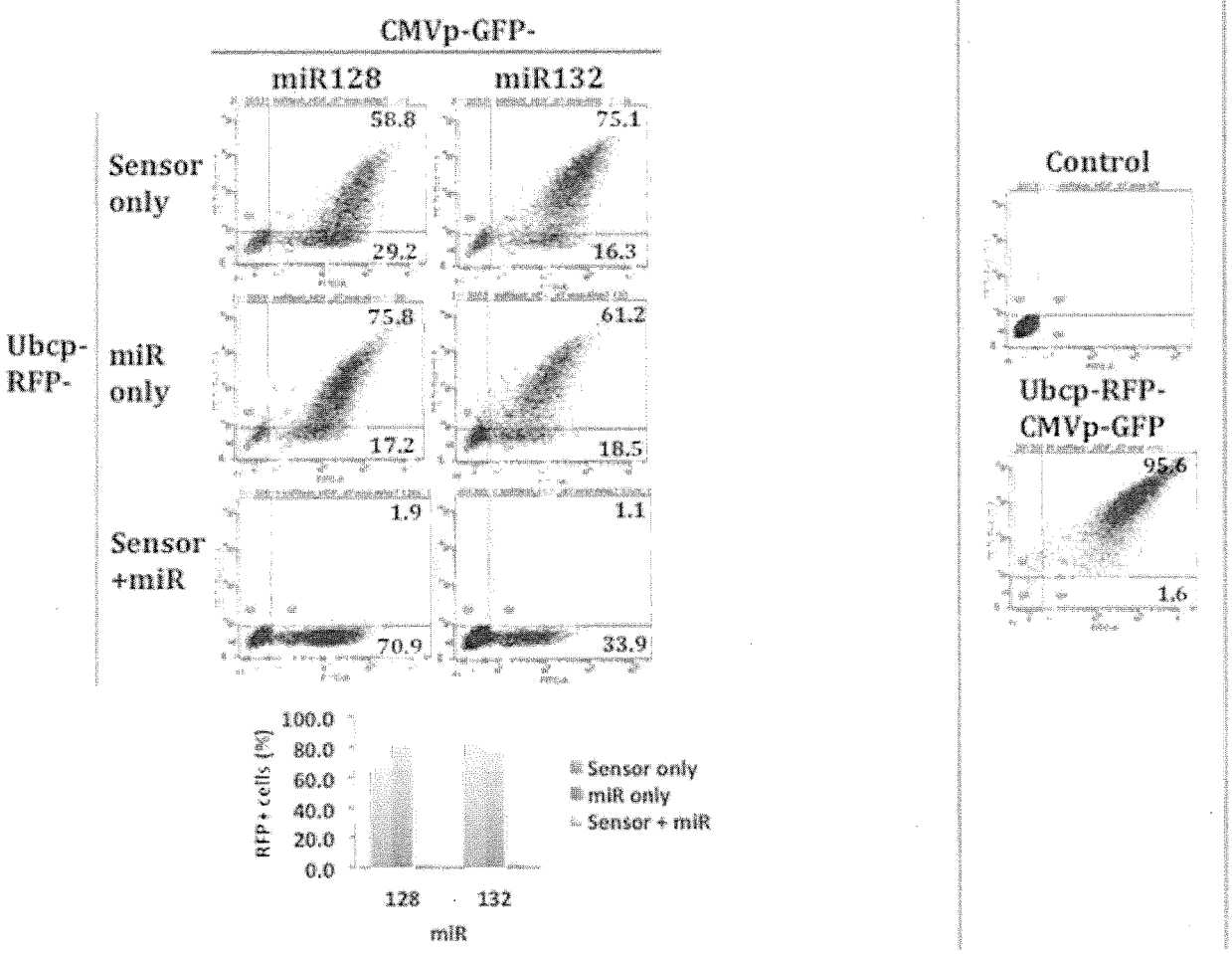

Sensor only
Ubcp
RFP
Sensor
CMVp
GFP
miR only
Ubcp
RFP
CMVp
GFP
miR
Sensor +miR
Ubcp
RFP
Sensor
CMVp
GFP
miR

# Figure 34

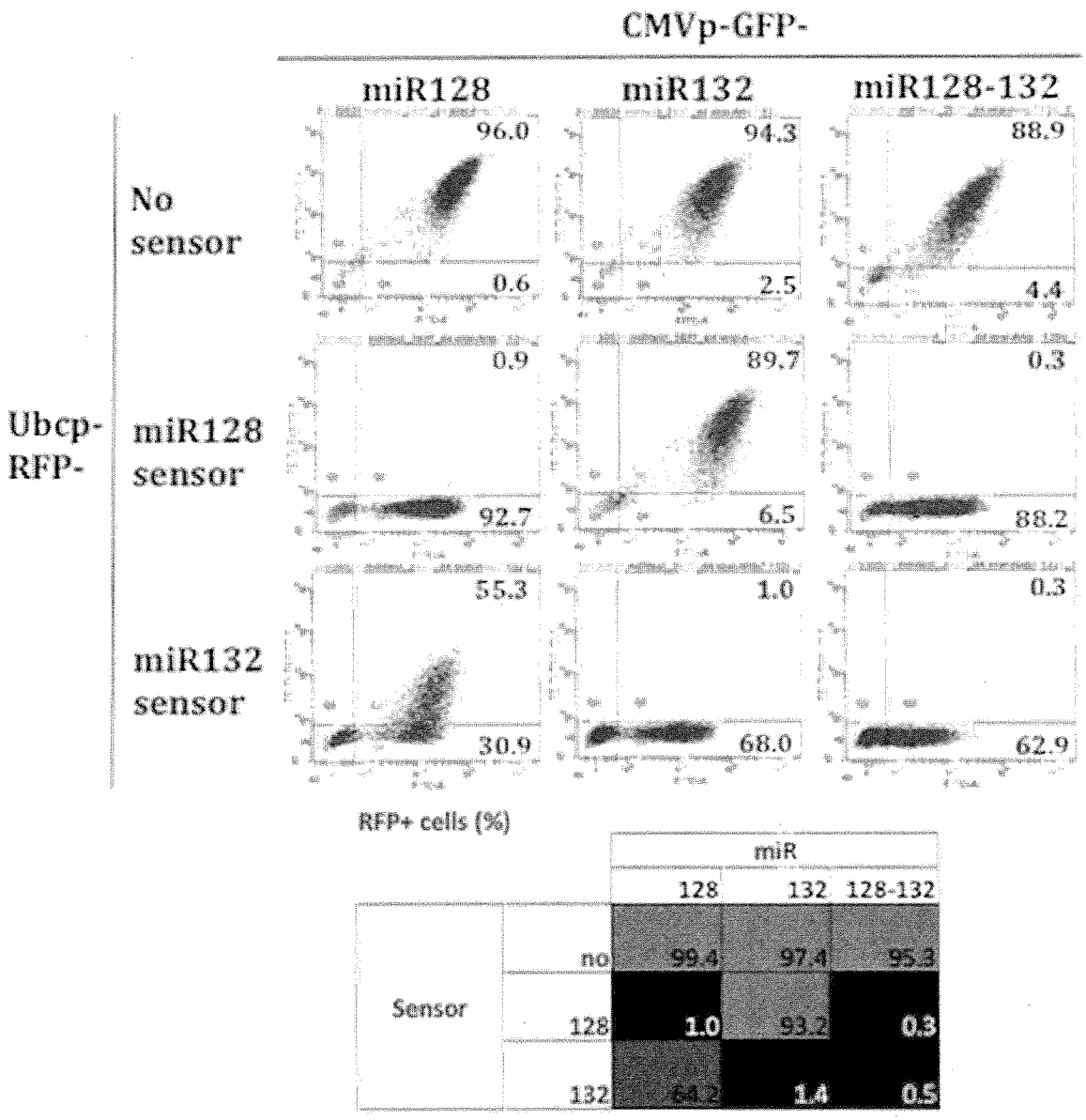

CMVp-GFP-
miR124
miR128
miR132
Ubcp-RFP-
Sensor only
miR only
Sensor +miR
99.6
0.1
95.2
4.4
96.7
2.0
79.0
15.8
96.0
0.6
94.3
2.5
1.7
86.1
0.9
92.7
1.0
68.0
RFP + cells (%)
100.0
80.0
60.0
40.0
20.0
0.0
124
128
132
miR
Sensor only
miR only
Sensor + miR


Control
Ubcp-RFP-
CMVp-GFP
99.7
0.1

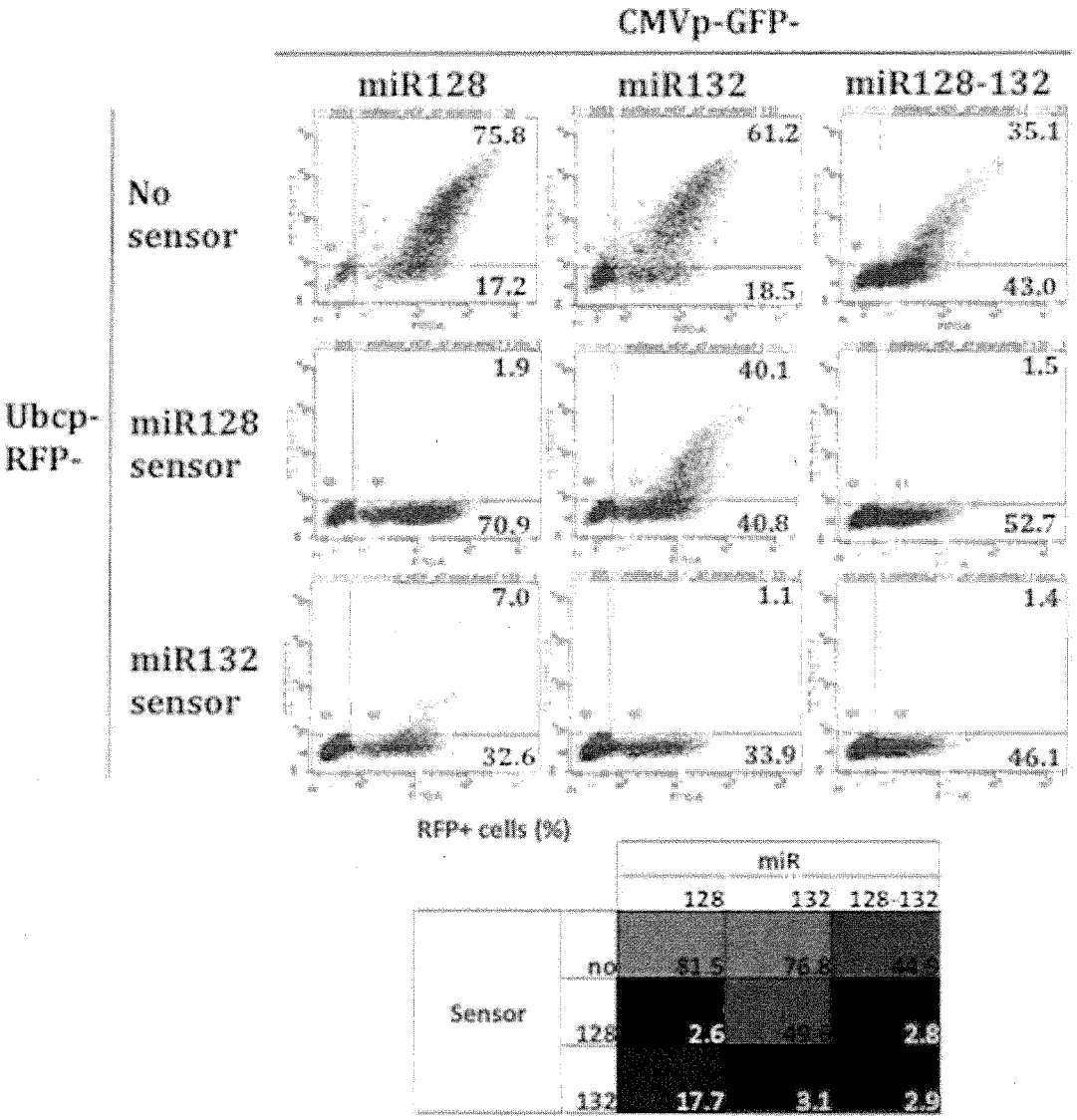
# Figure 35


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
Control
Ubcp-RFP-CMVp-GFP
95.6
1.6
RFP+ cells (%)
100.0
80.0
60.0
40.0
20.0
0.0
128
132
miR
Sensor only
miR only
Sensor + miR

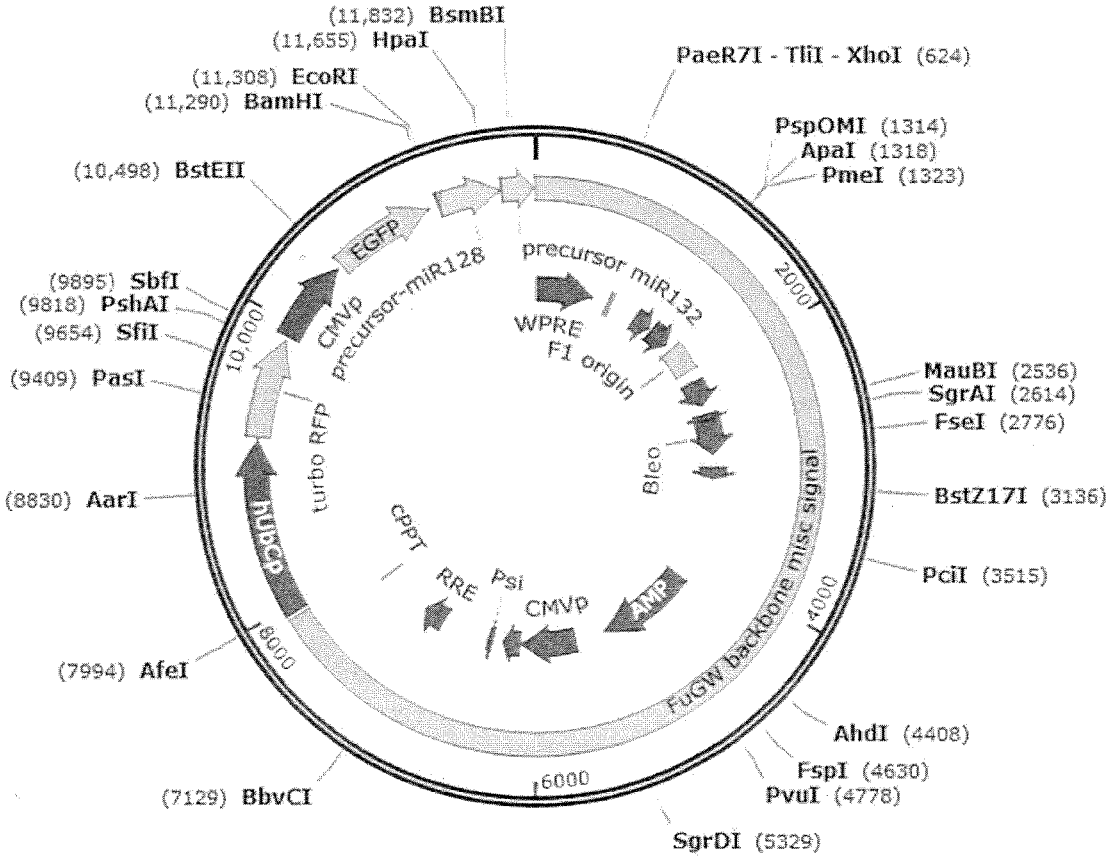
# Figure 36


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
99.4
97.4
95.3
1.0
93.2
0.3
64.2
1.4
0.5

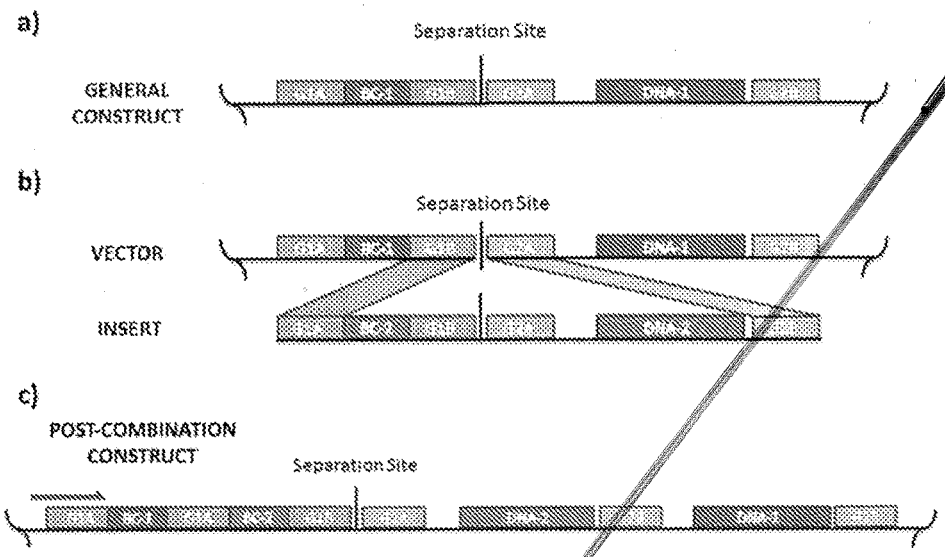
# Figure 37


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
81.5
76.8
2.6
2.8
17.7
3.1
2.9

# Figure 38


(11,832) BsmBI
(11,655) HpaI
(11,308) EcoRI
(11,290) BamHI
(10,498) BstEII
(9895) SbfI
(9818) PshAI
(9654) SfiI
(9409) PasI
(8830) 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-miR128
precursor miR132
WPRE
F1 origin
Bleo
turbo RFP
cPPT
RRE
Psi
CMVp
AMP
FuGW backbone misc signal
2000
4000
6000
8000
10,000

# Figure 1


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

# Figure 2

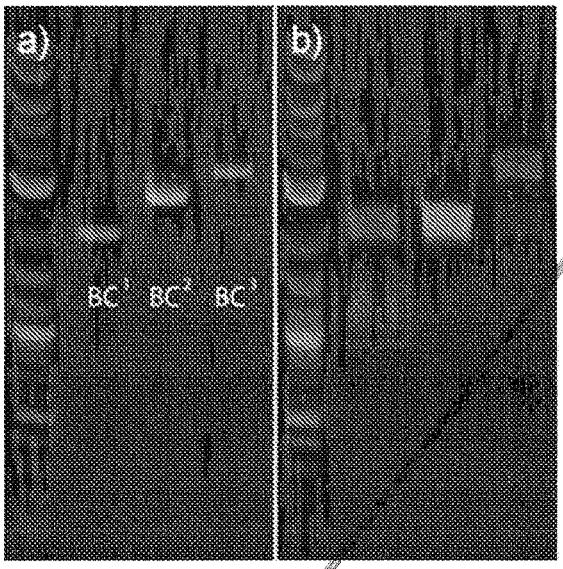
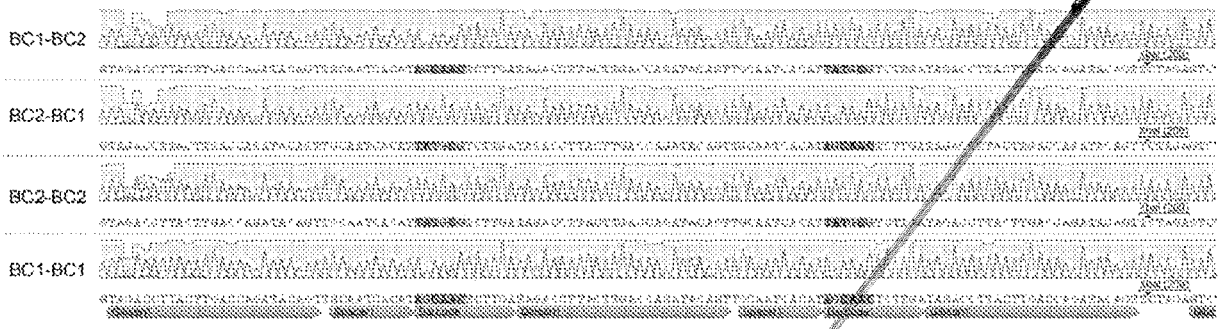


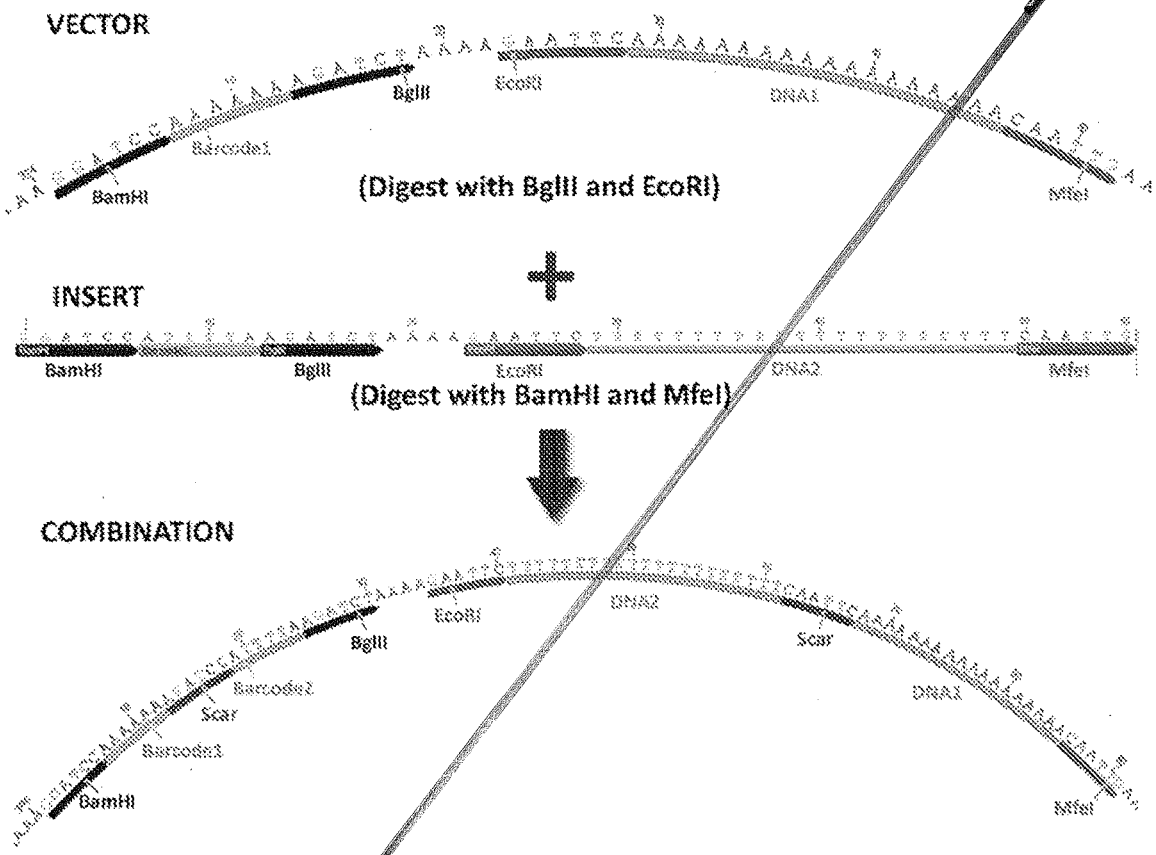
Figure 3



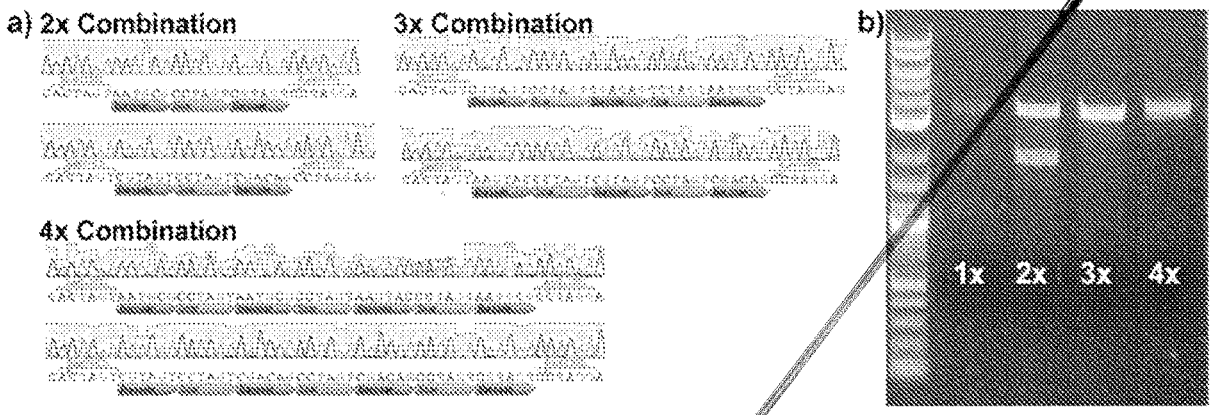
# Figure 4



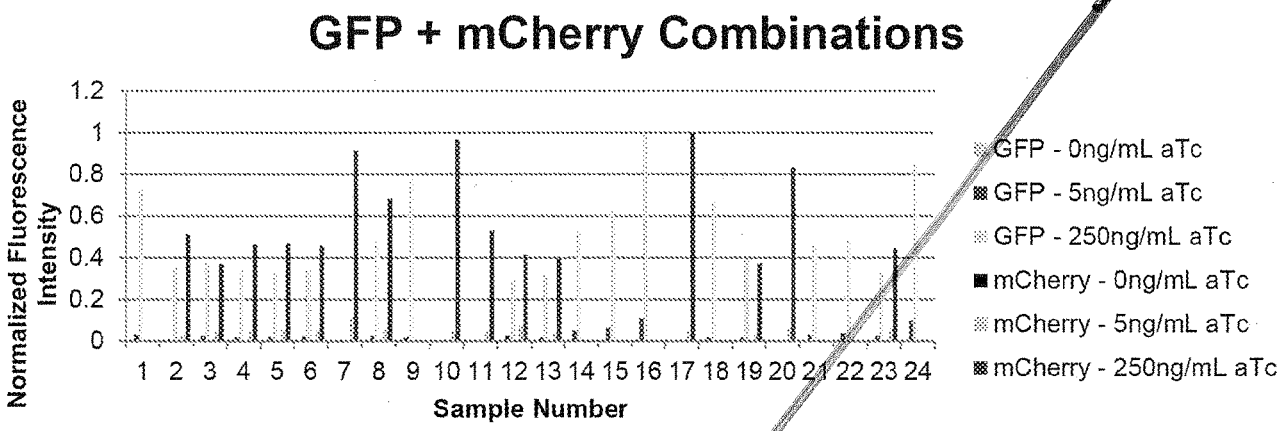
# Figure 5


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

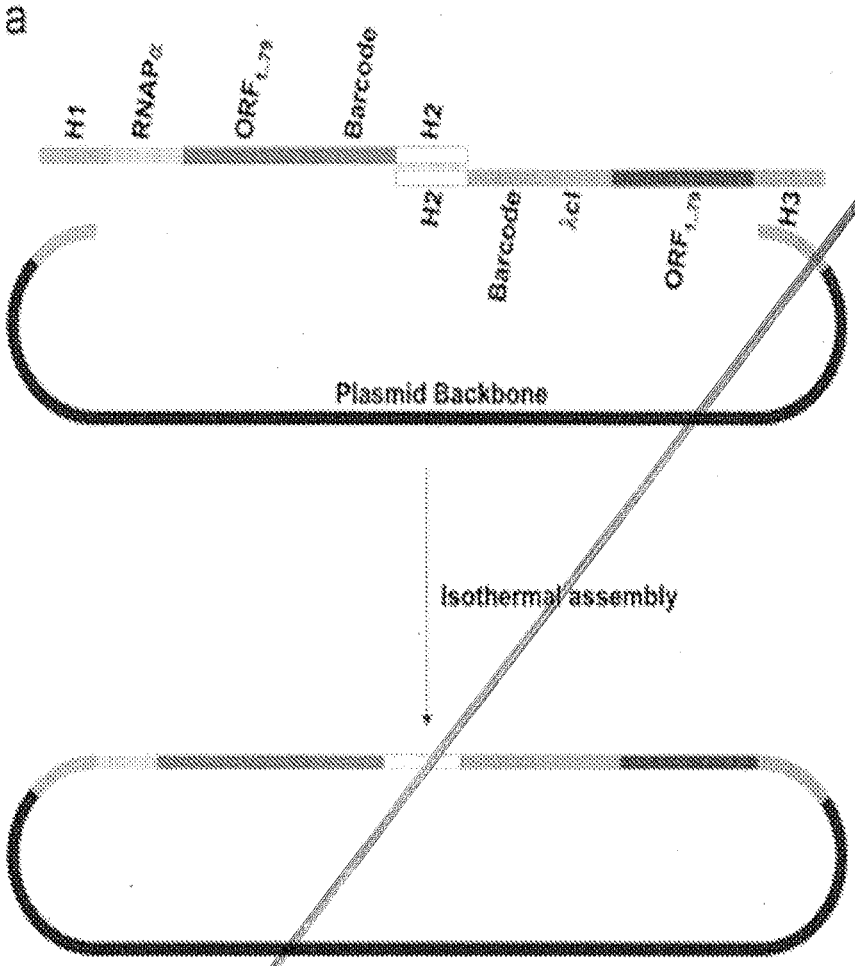
# Figure 6



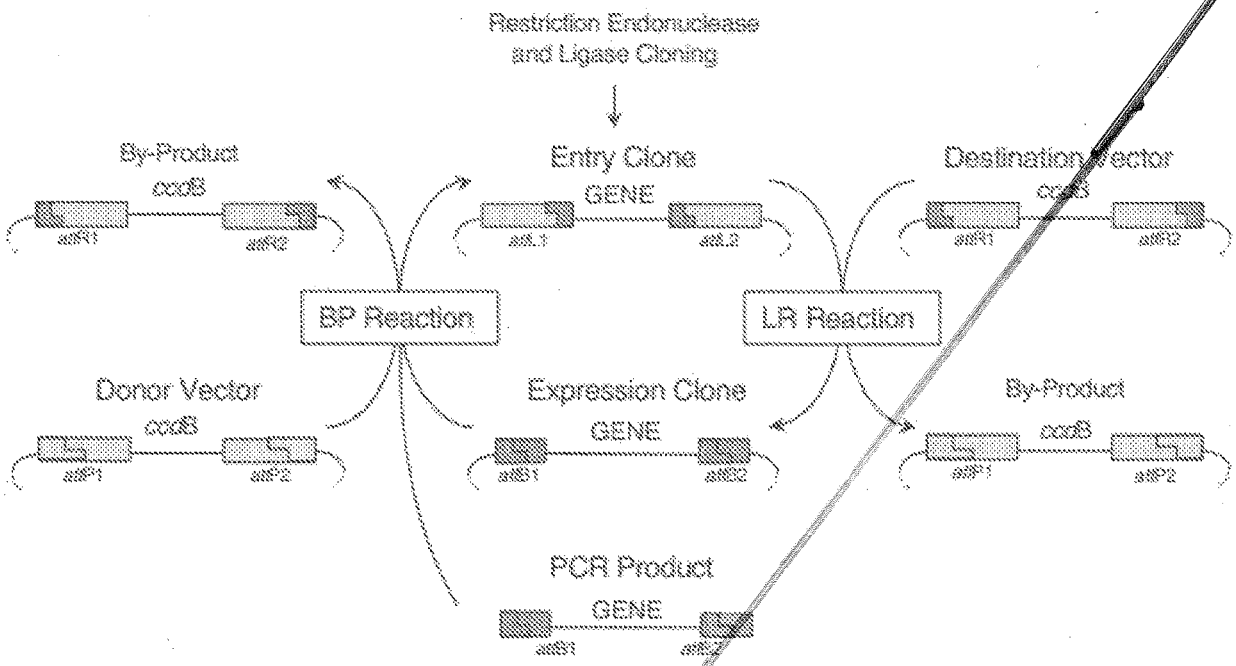
# Figure 7


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


H1
RNAP
ORF
Barcode
H2
H2
Barcode
Act
ORF
H3
Plasmid Backbone
Isothermal assembly

# Figure 9



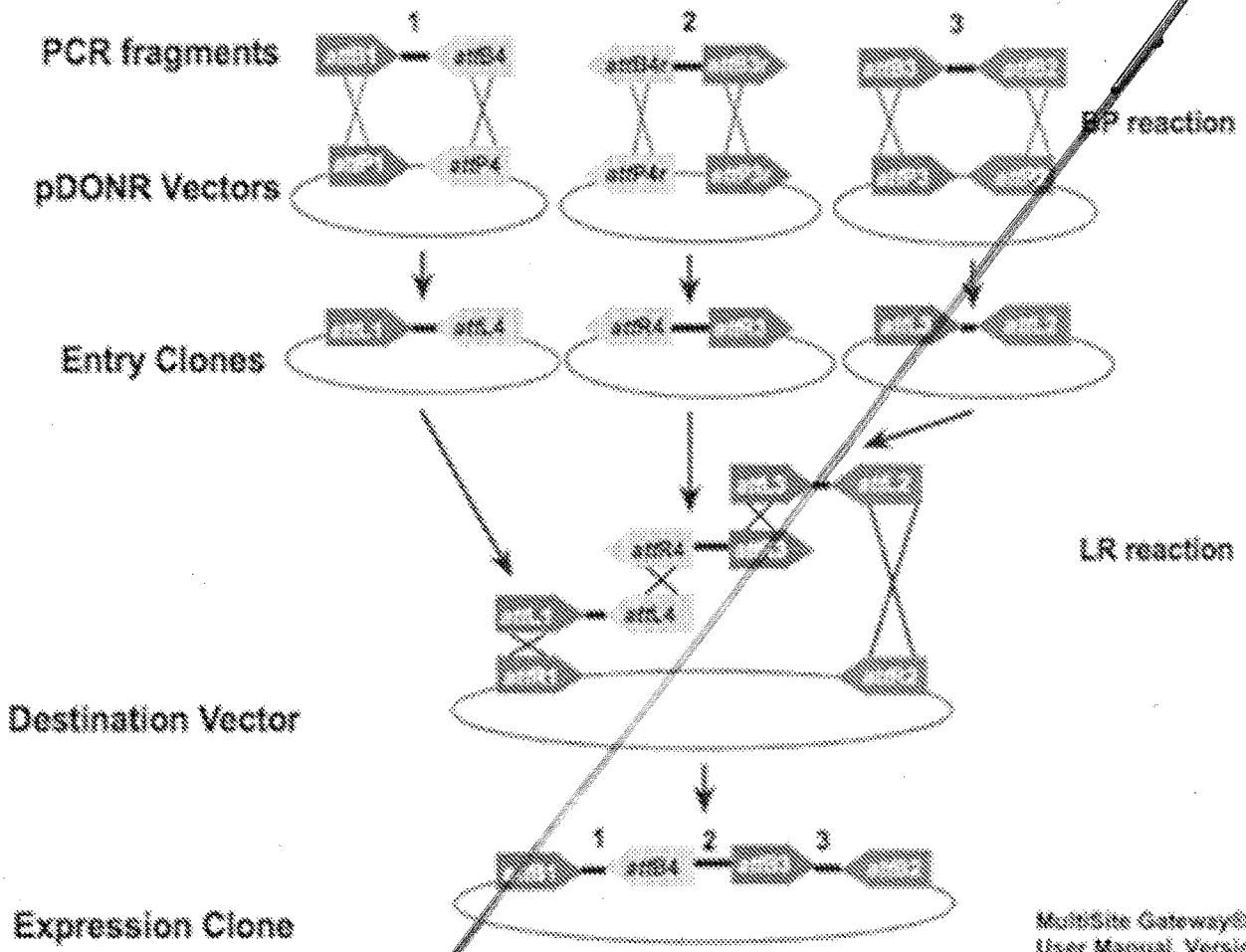

Key:

B1__*__B2 + P1_____P2

L1__*__L2 + R1_____R2

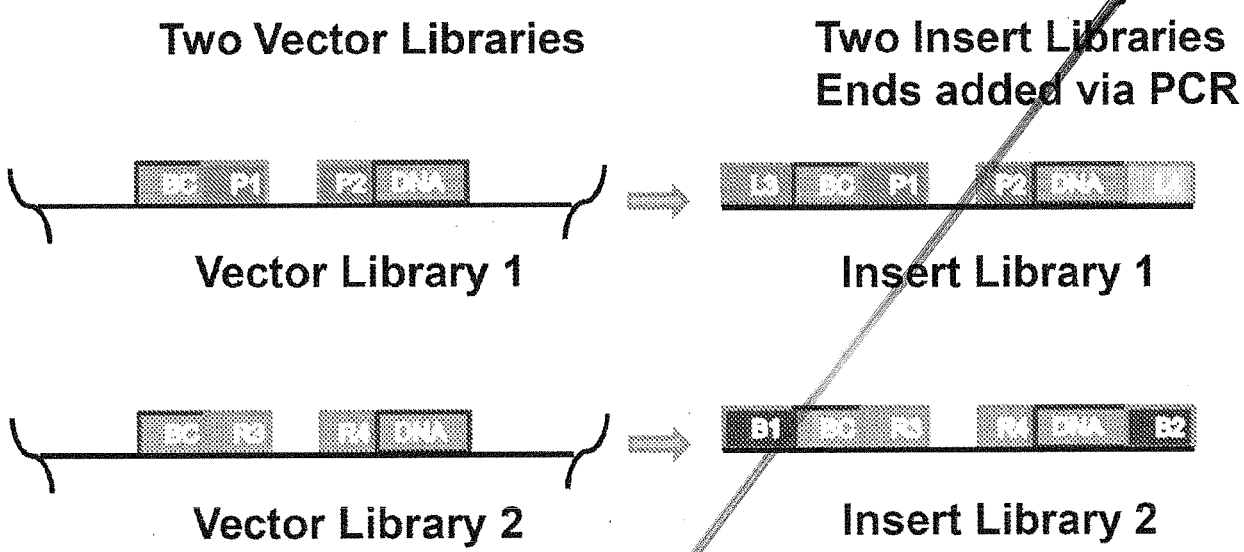
http://wolfson.huji.ac.il/expression/gatewayman.pdf

# 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

12/35

## Figure 12

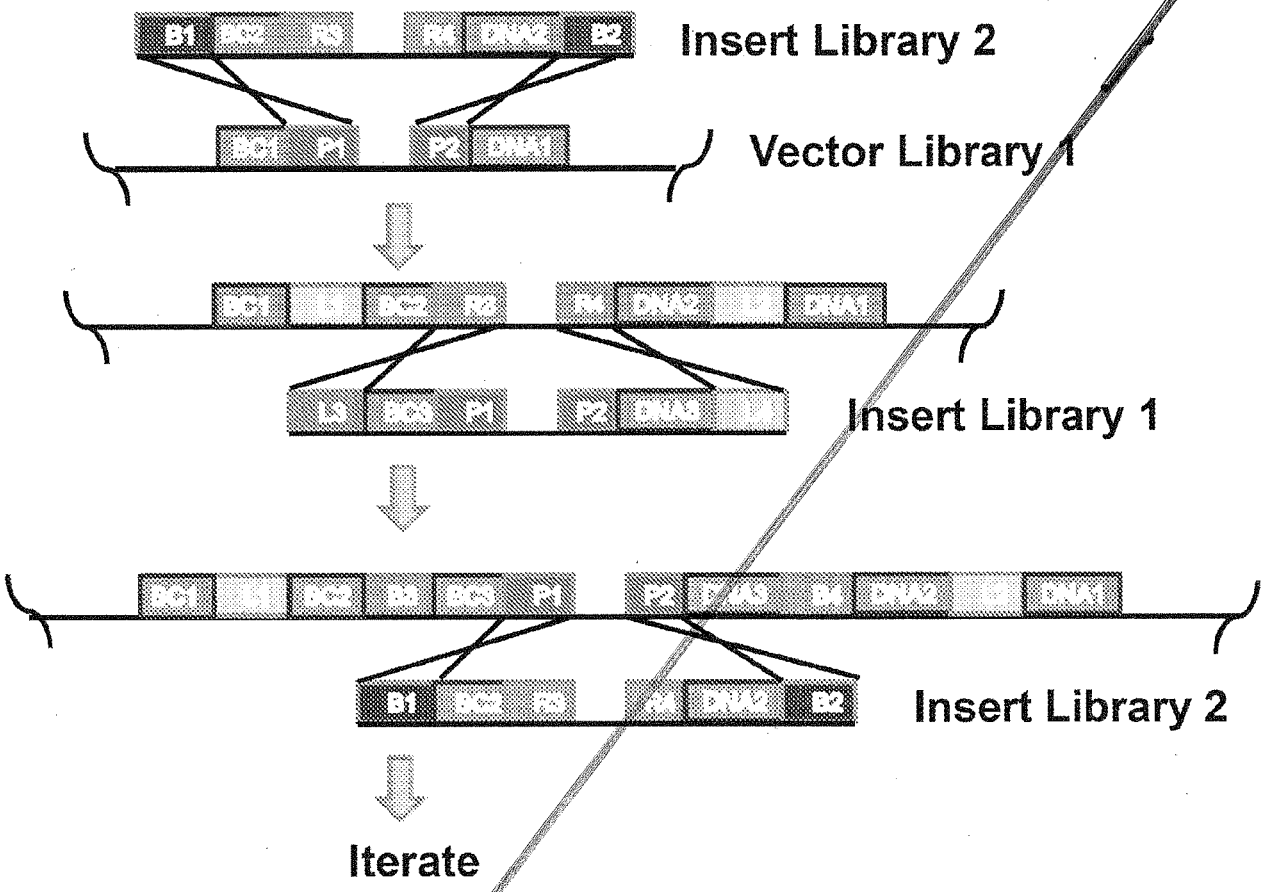
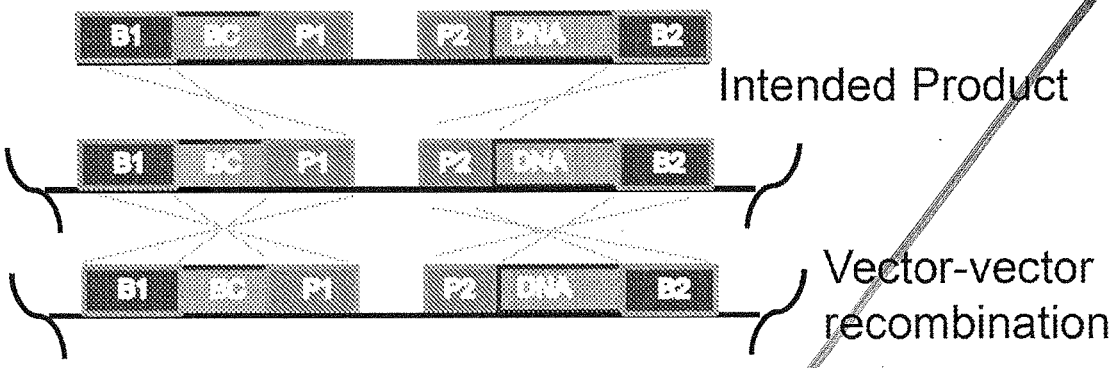
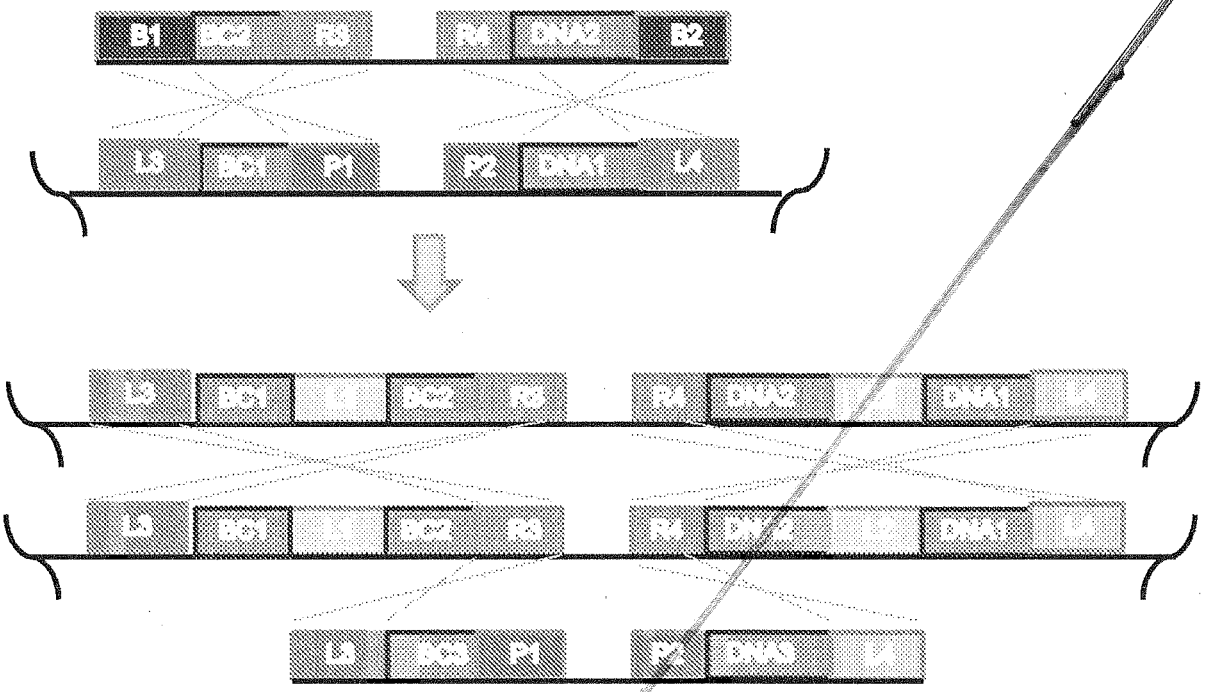


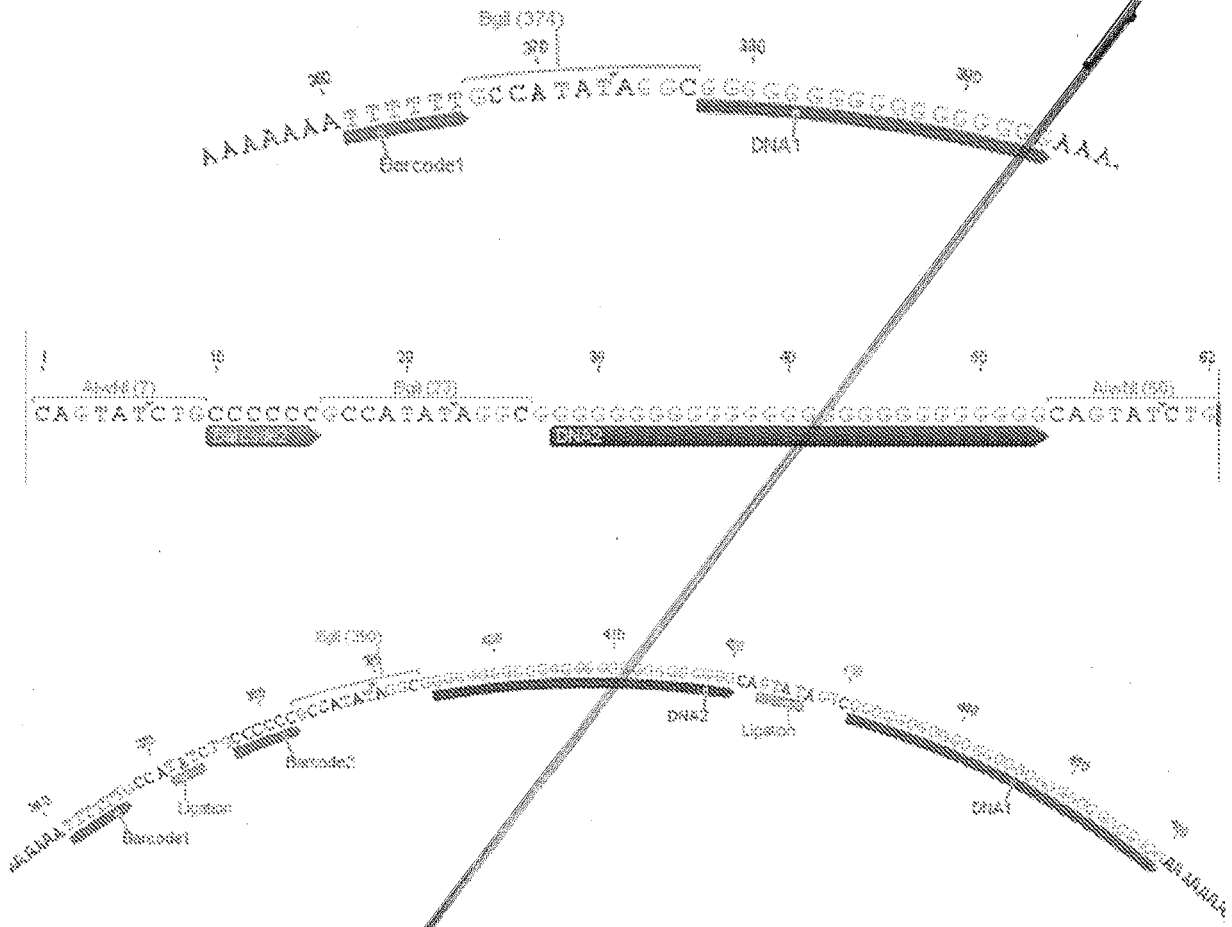
**Figure 13**


B1
P1
P2
DNA
B2
Intended Product
Vector-vector
recombination

# Figure 14



# Figure 15



# Figure 16

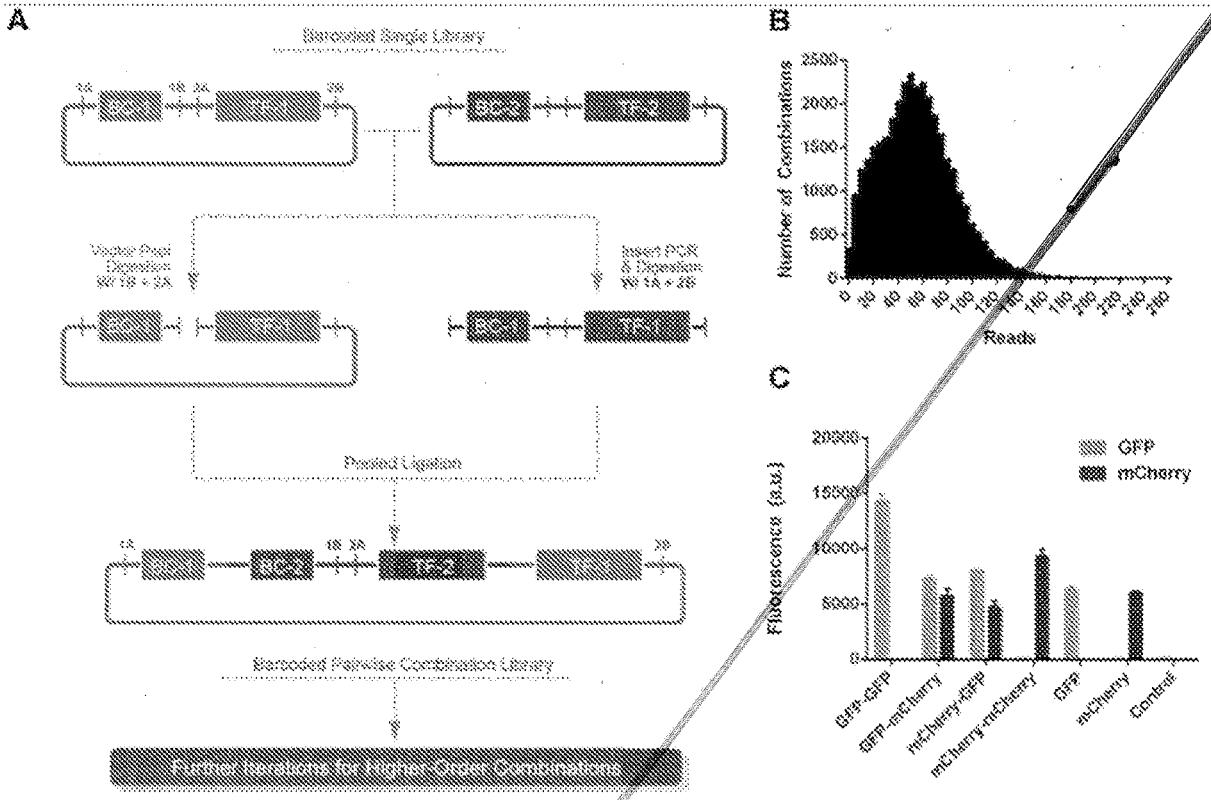

A
Barcoded Single Library
Pooled Ligation
Barcoded Pairwise Combination Library
B
Number of Combinations
Reads
C
Fluorescence (a.u.)
GFP
mCherry
GFP-GFP
GFP-mCherry
mCherry-GFP
mCherry-mCherry
GFP
mCherry
Control

**Figure 17**

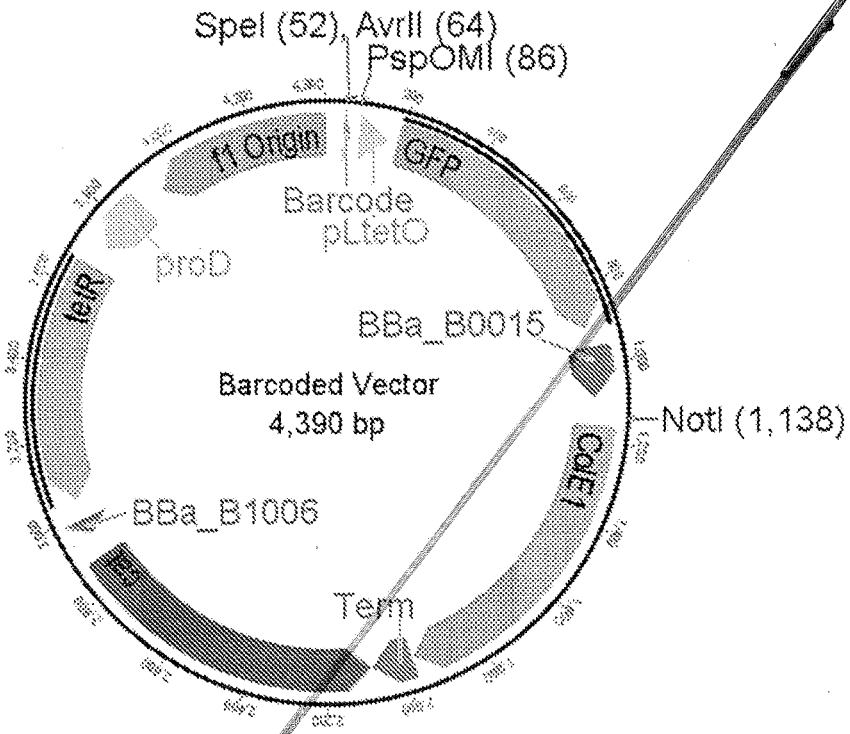
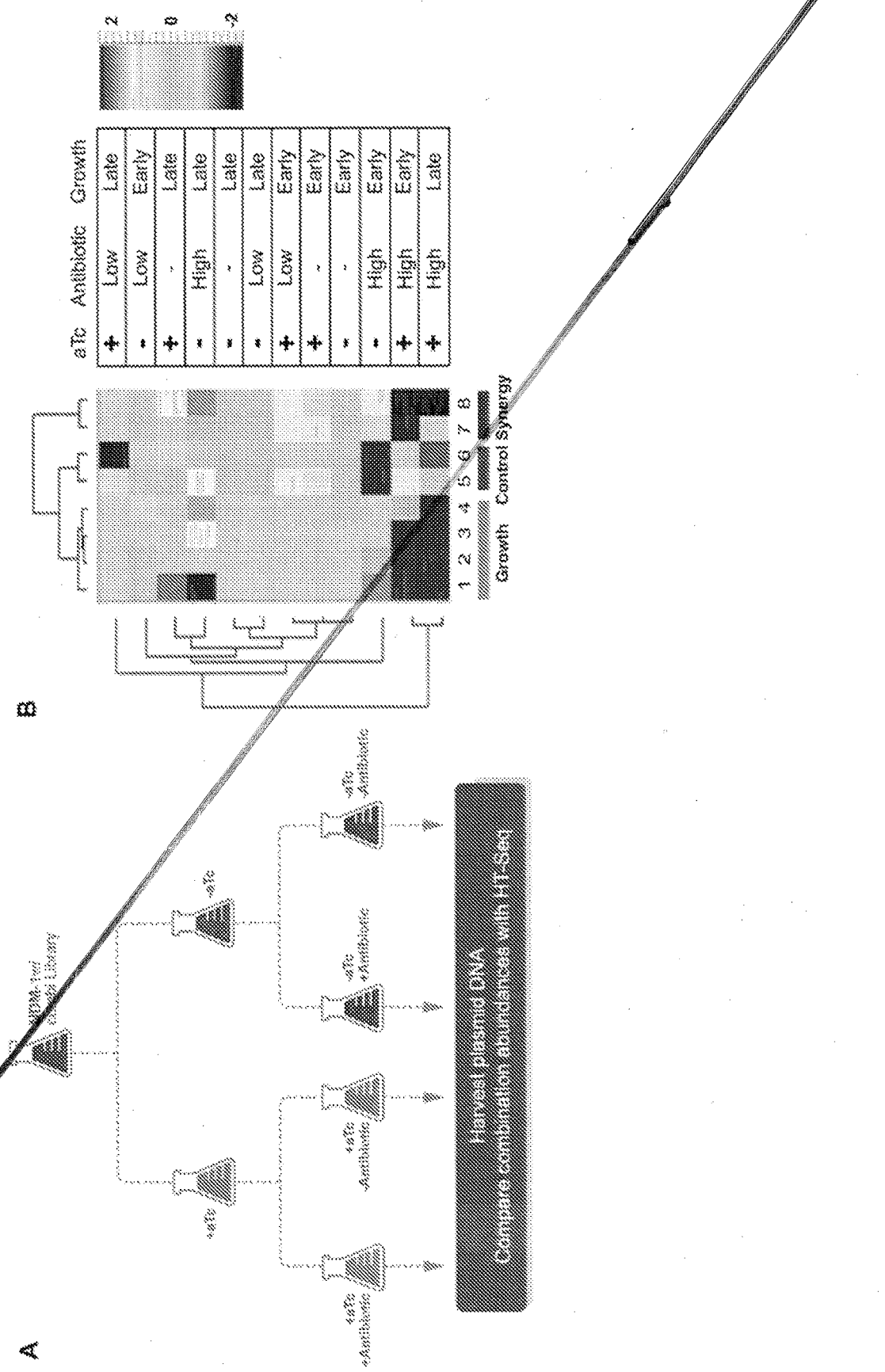
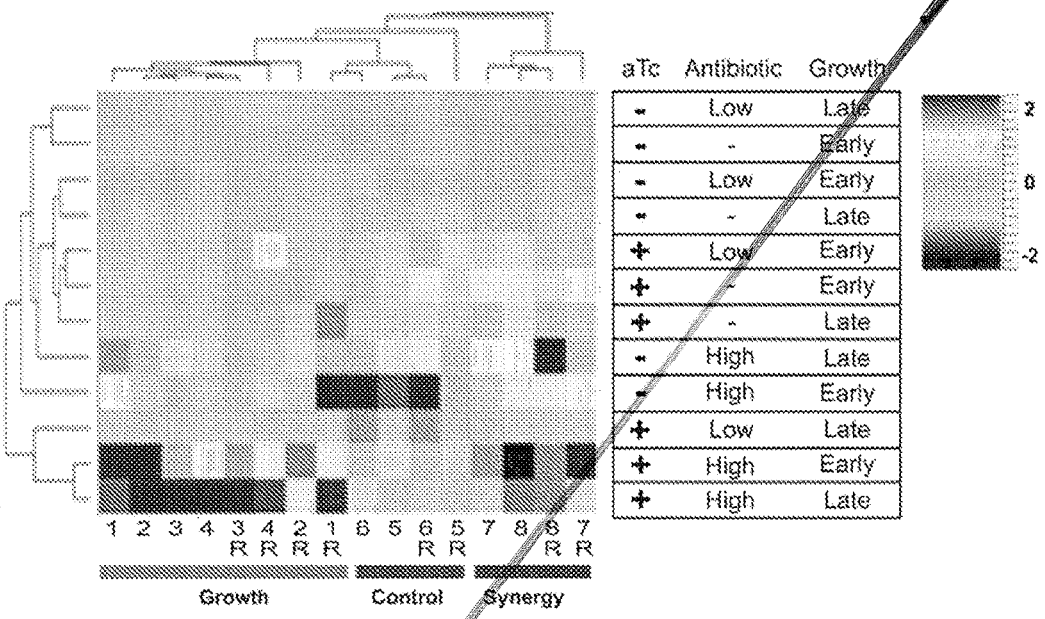

SpeI (52), AvrII (64)
PspOMI (86)
f1 Origin
GFP
Barcode
pLtetO
proD
tetR
BBa_B0015
Barcoded Vector
4,390 bp
NotI (1,138)
ColE1
BBa_B1006
Kan
Term

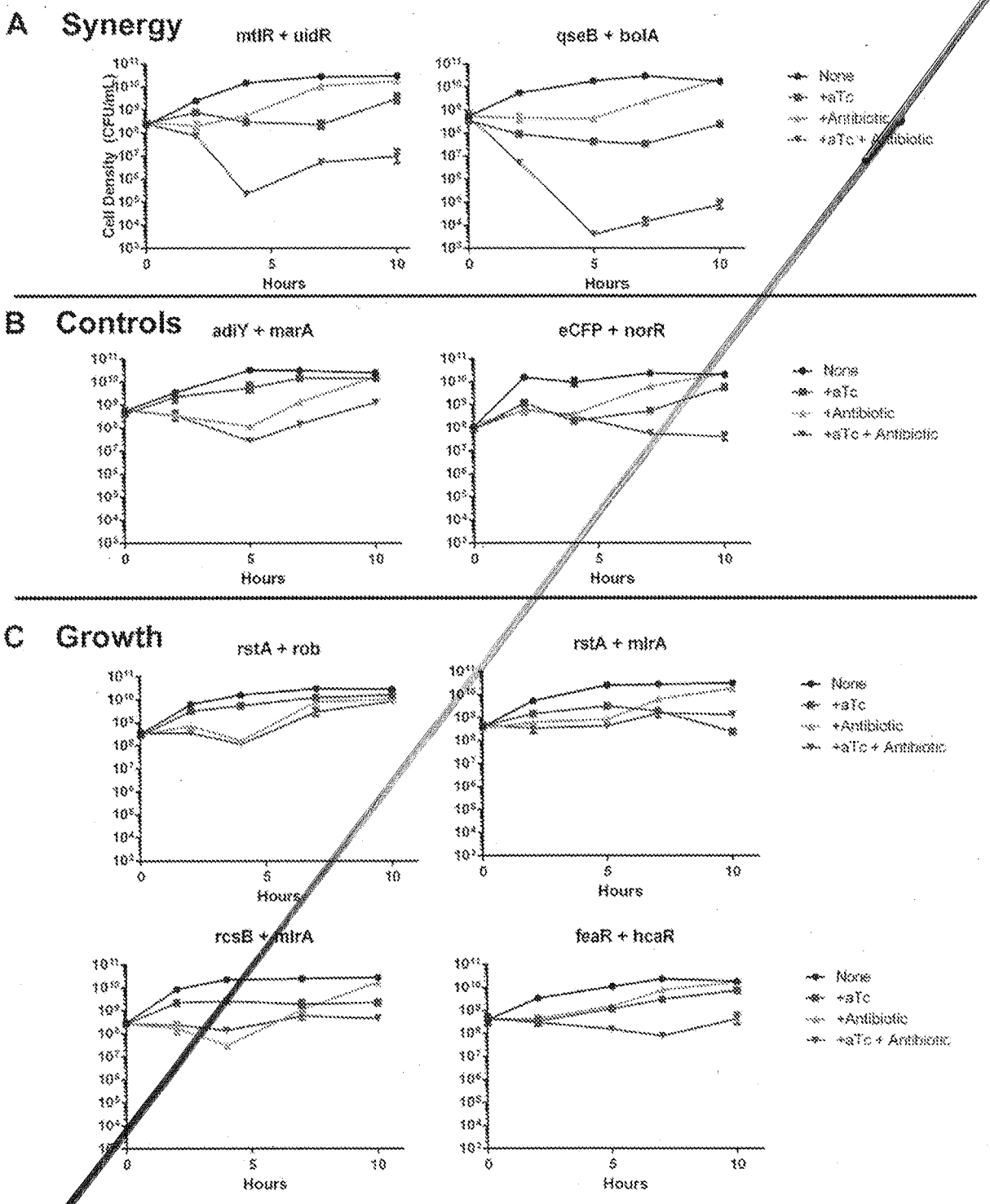
Figure 18


A
Harvest plasmid DNA
Capture combination dependences with HTS Seq
B
aTc
Antibiotic
Growth
Growth
Control Synergy

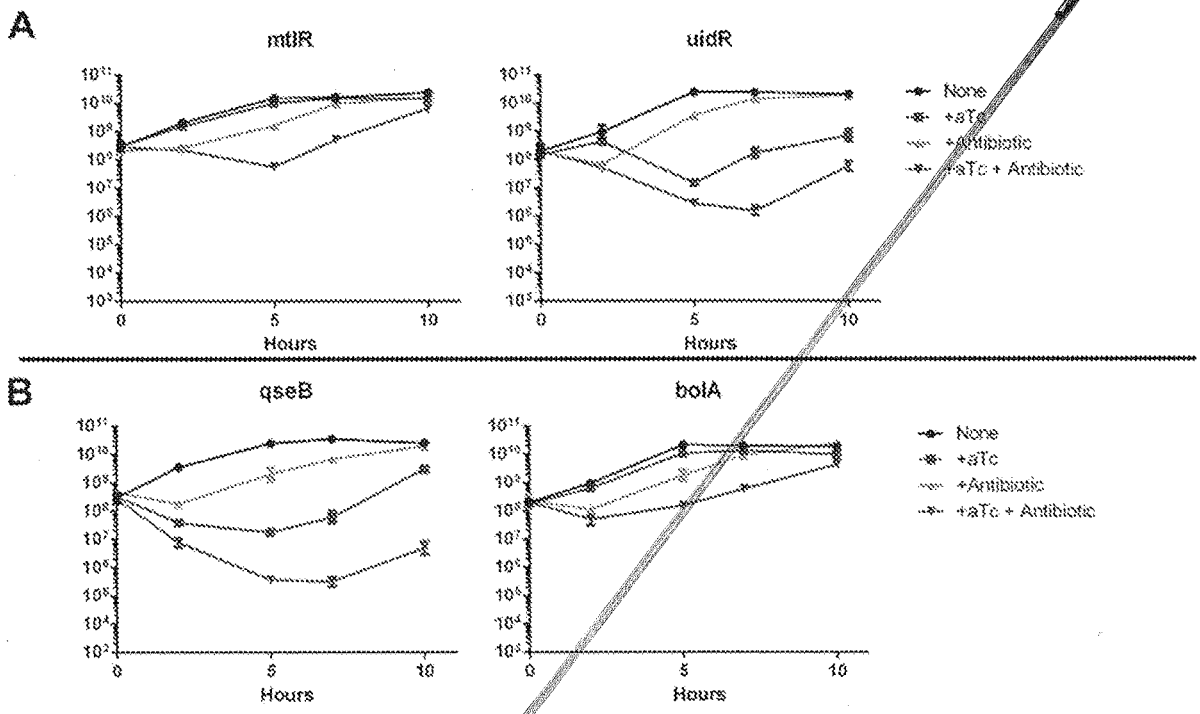
# Figure 19


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 9R 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

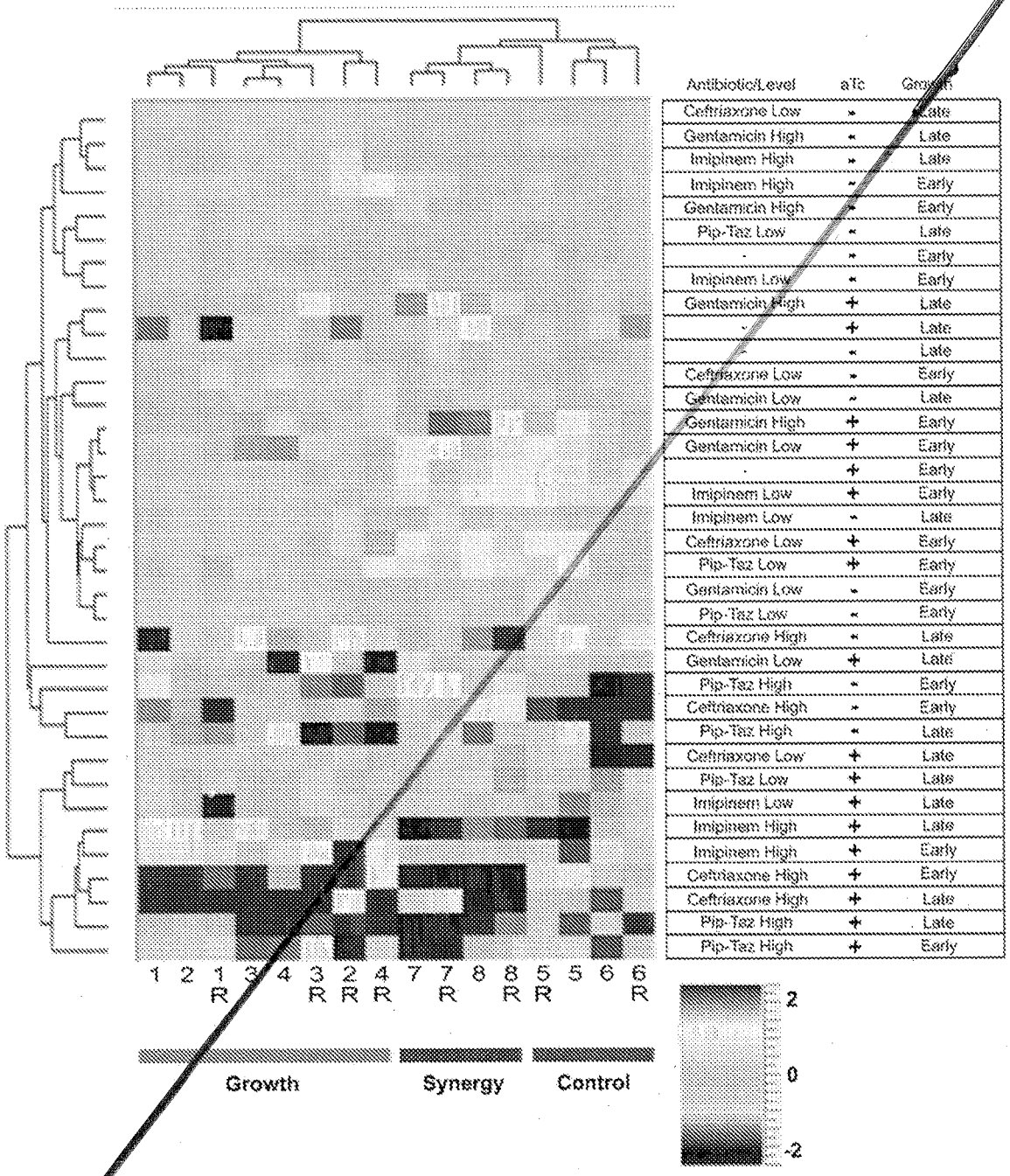
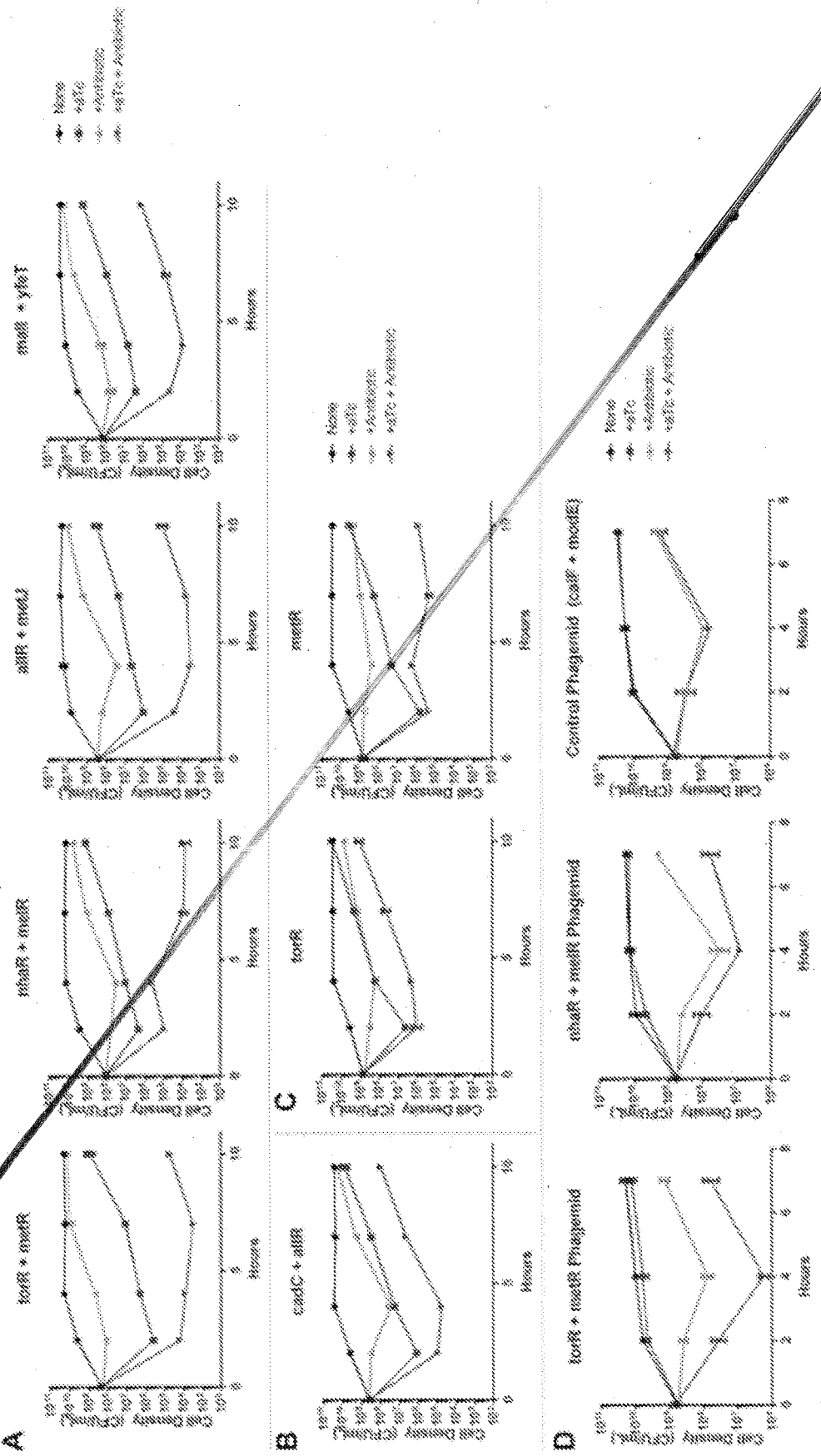
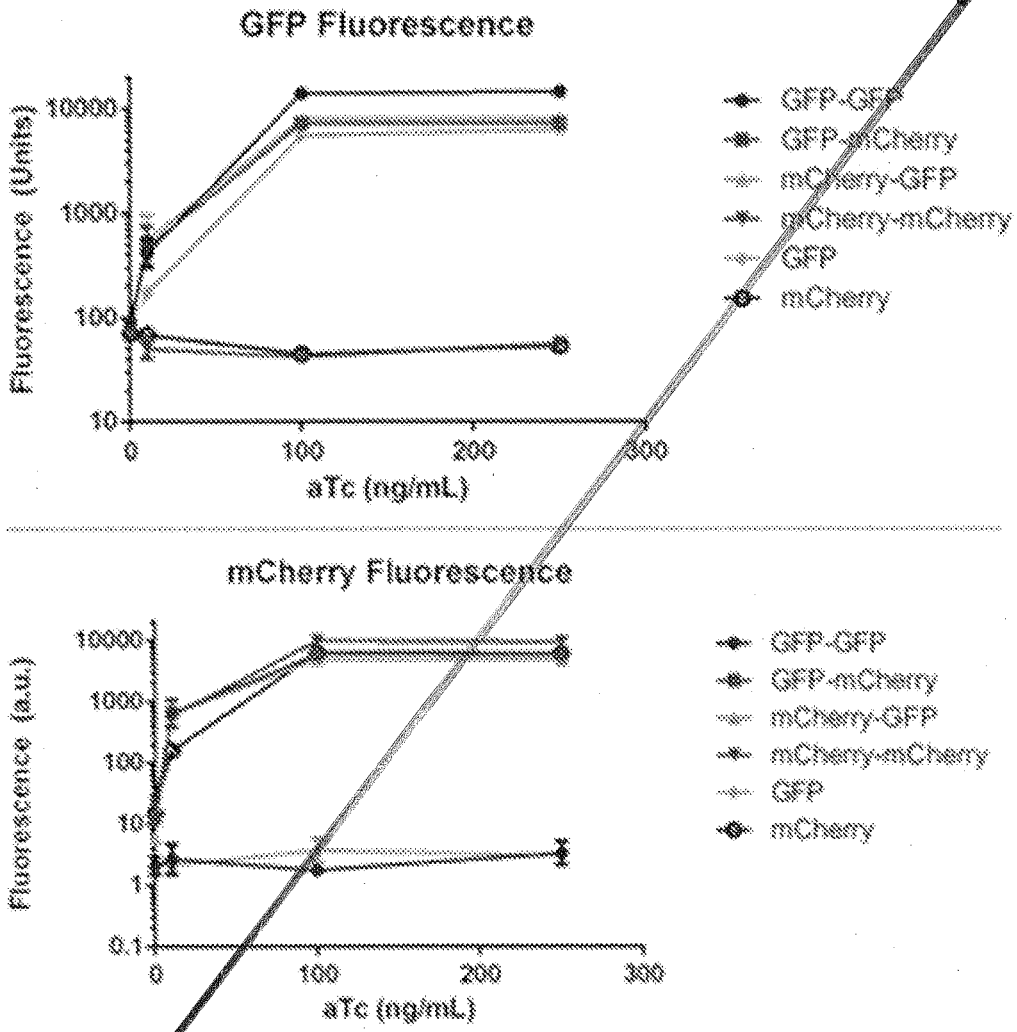

Antibiotic/Level
aTc
Growth
Ceftriaxone Low - Late
Gentamicin High - Late
Imipinem High - Late
Imipinem High - Early
Gentamicin High - Early
Pip-Taz Low - Late
- - Early
Imipinem Low - Early
Gentamicin High + Late
- + Late
- - Late
Ceftriaxone Low - Early
Gentamicin Low - Late
Gentamicin High + Early
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 + mefA
tetR + mefA
tetR + mefA Plasmid
tetR + mefA Plasmid
cadC + ampR
tetR
tetR
Control Plasmid (cadC + mefA)
ampR + mefA
ampR + mefA
None
+ pTc
+ Antibiotic
+ pTc + 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

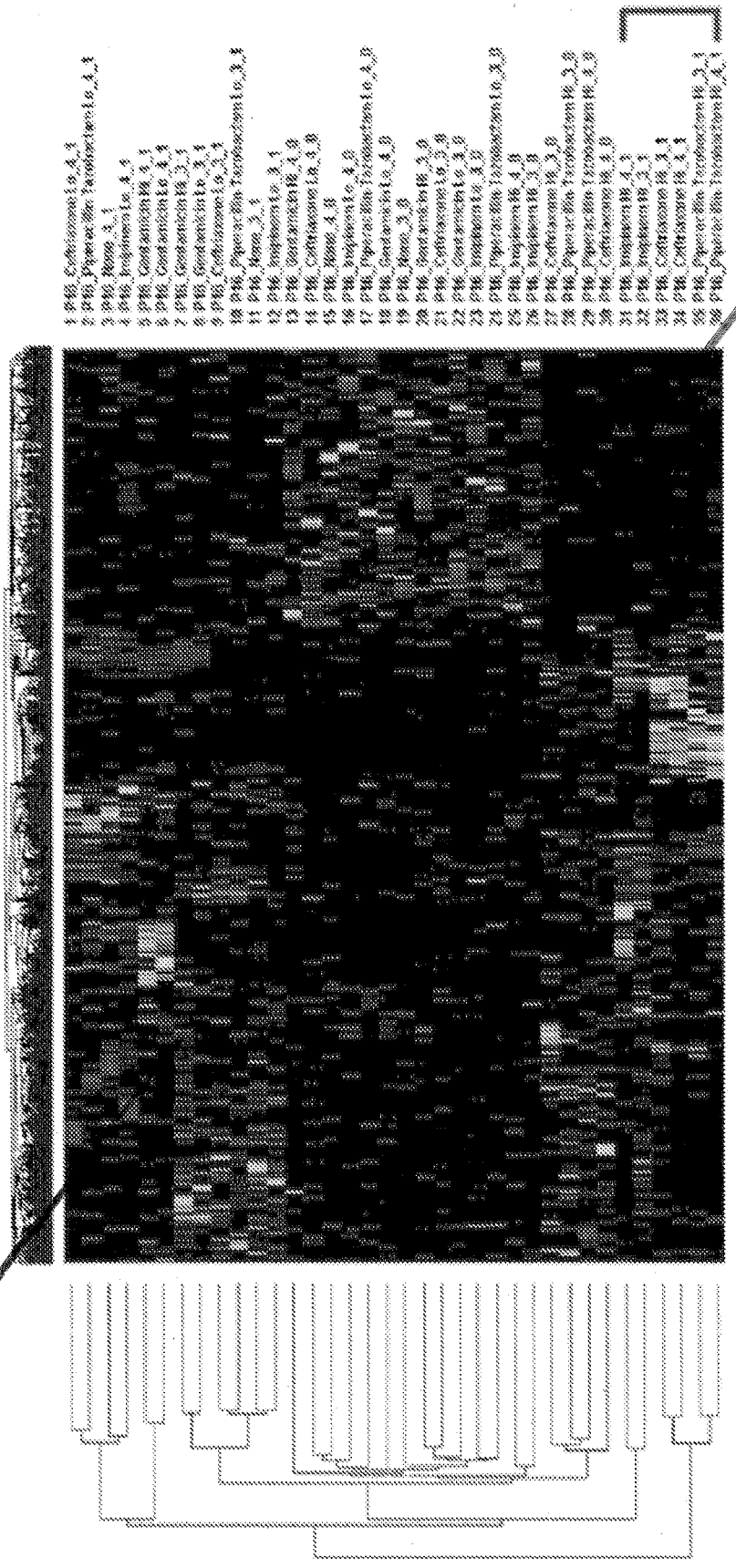


Figure 25

Figure 26

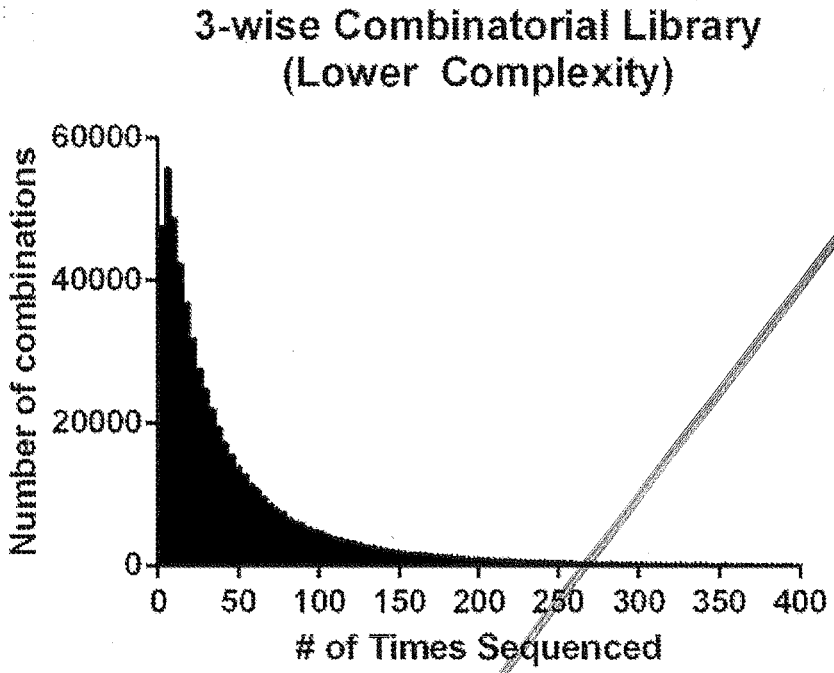



Figure 27

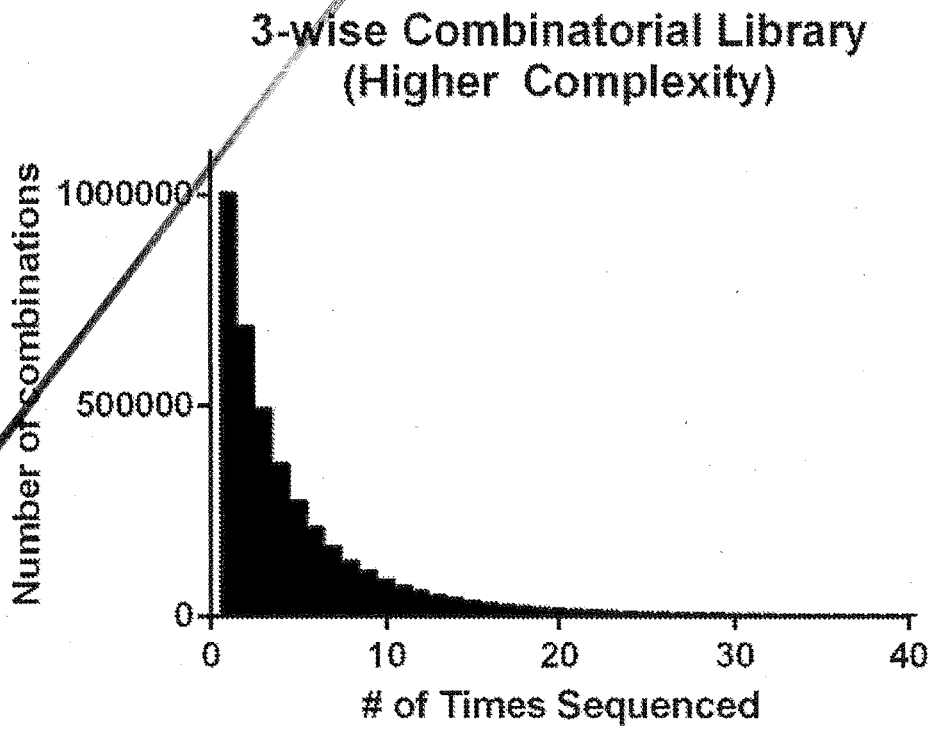
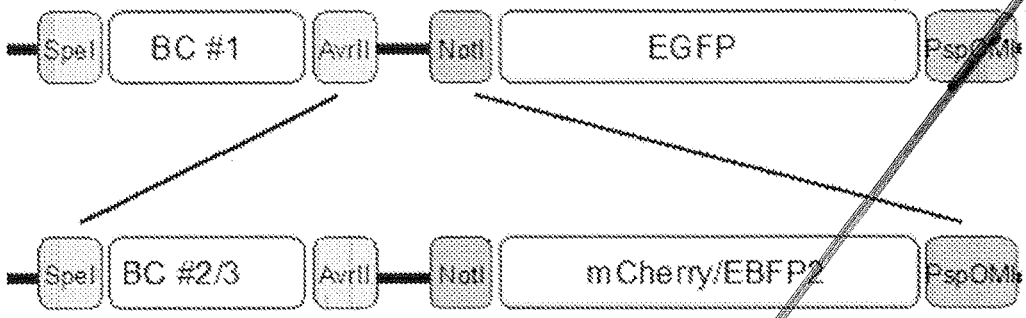


Figure 28


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

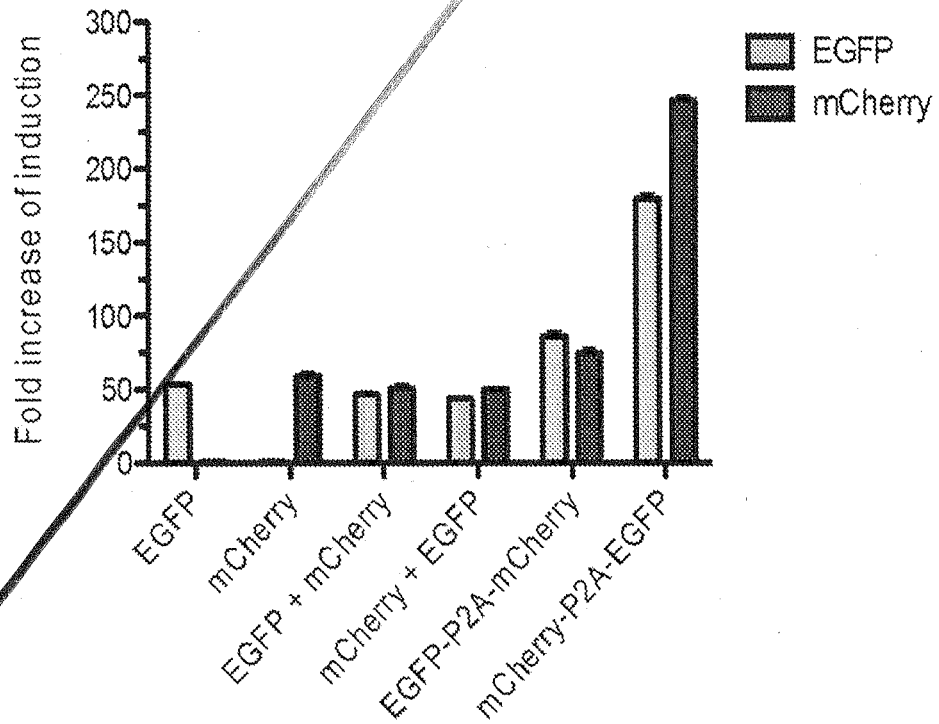


**Figure 31**

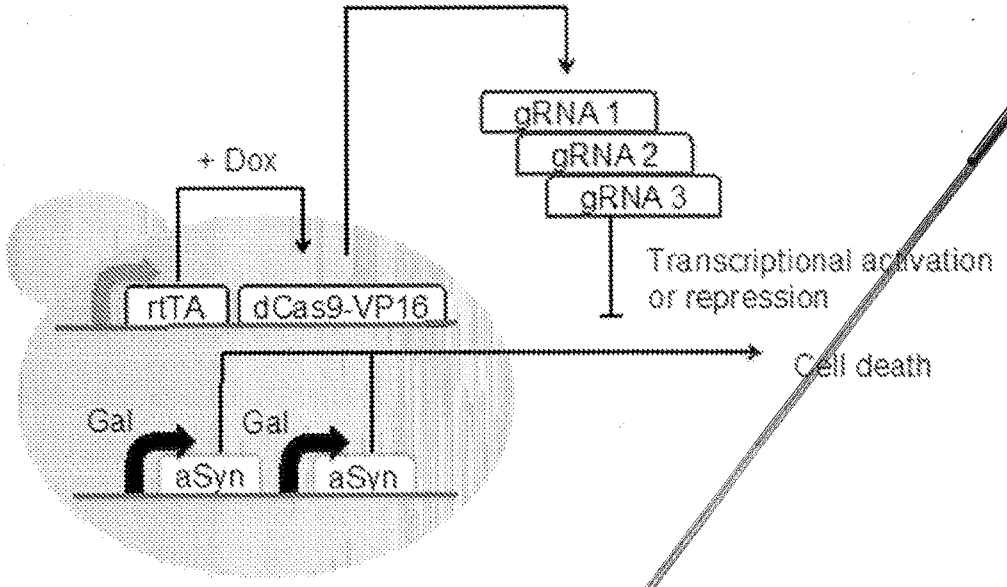
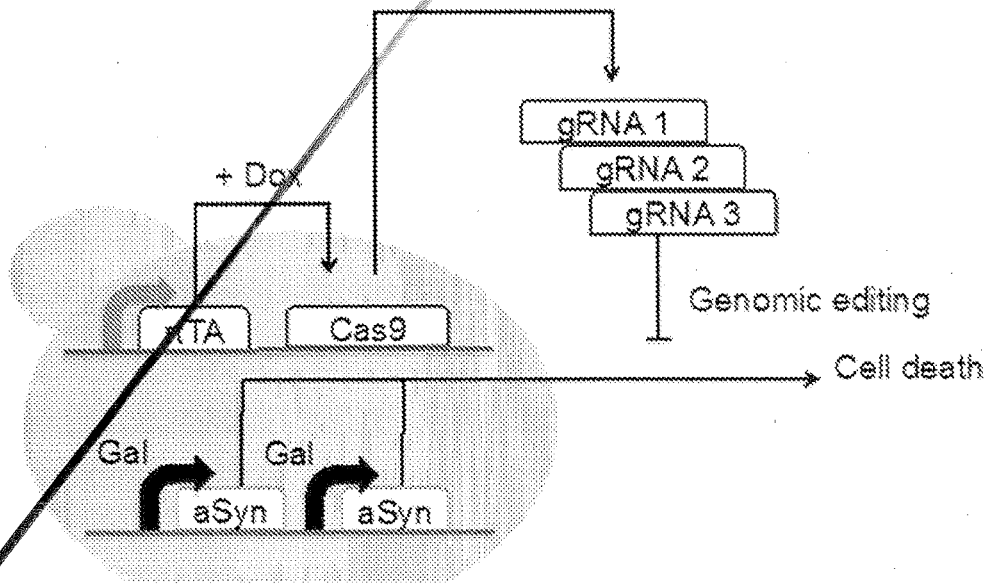
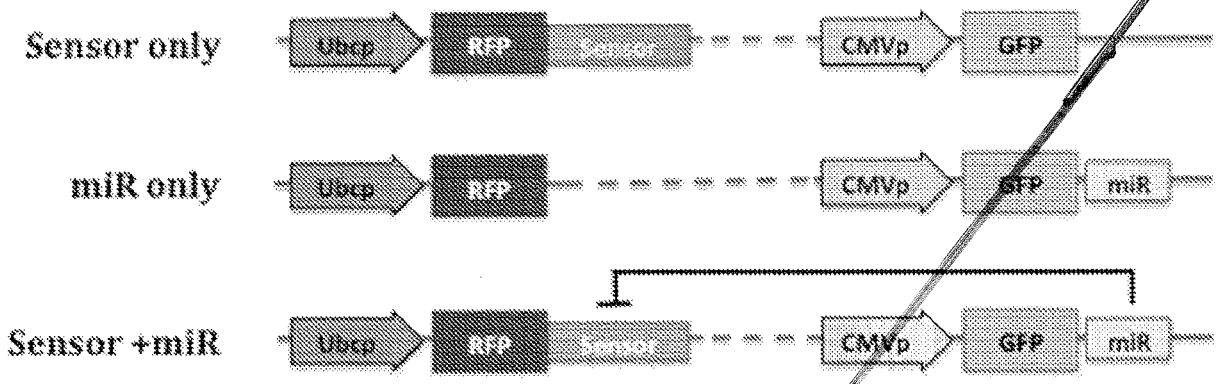


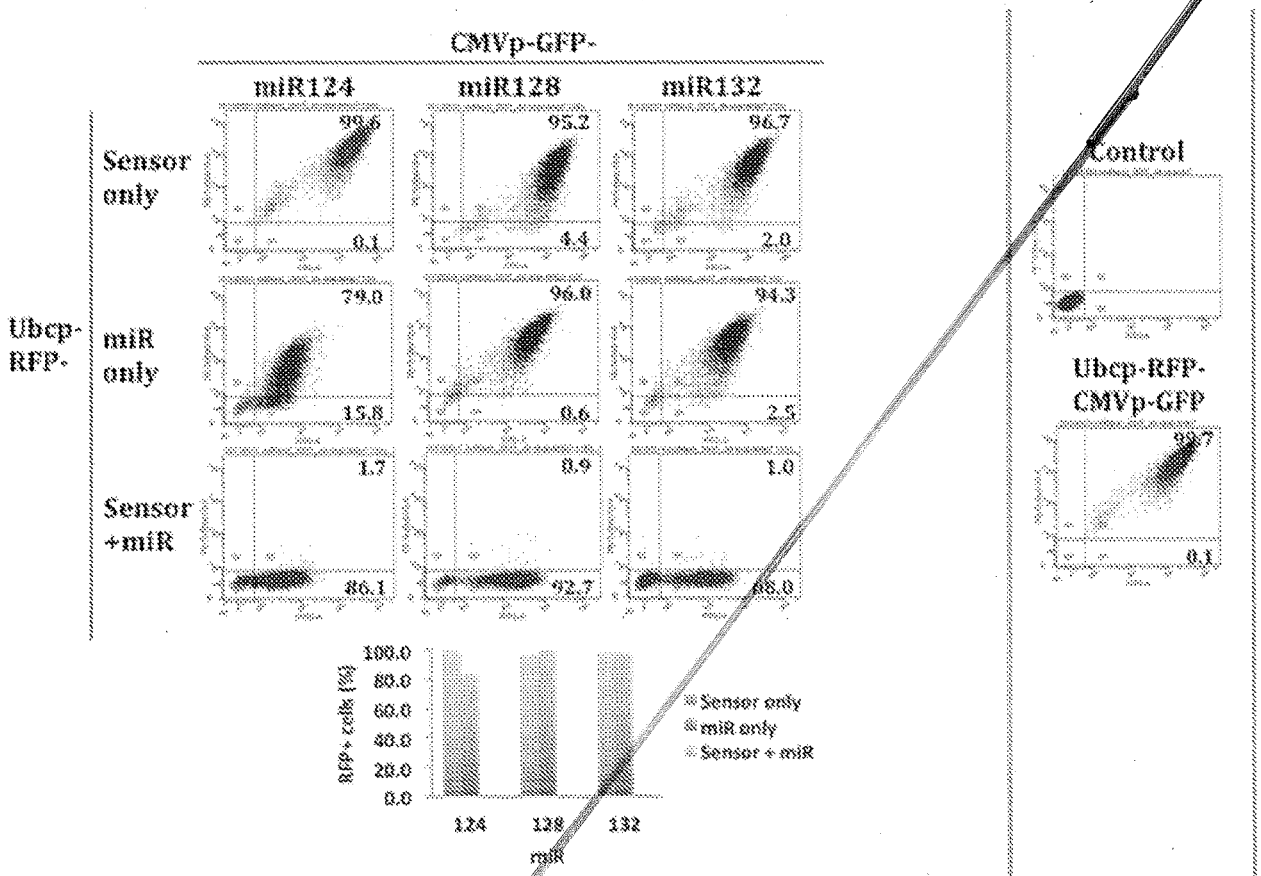

**Figure 32**



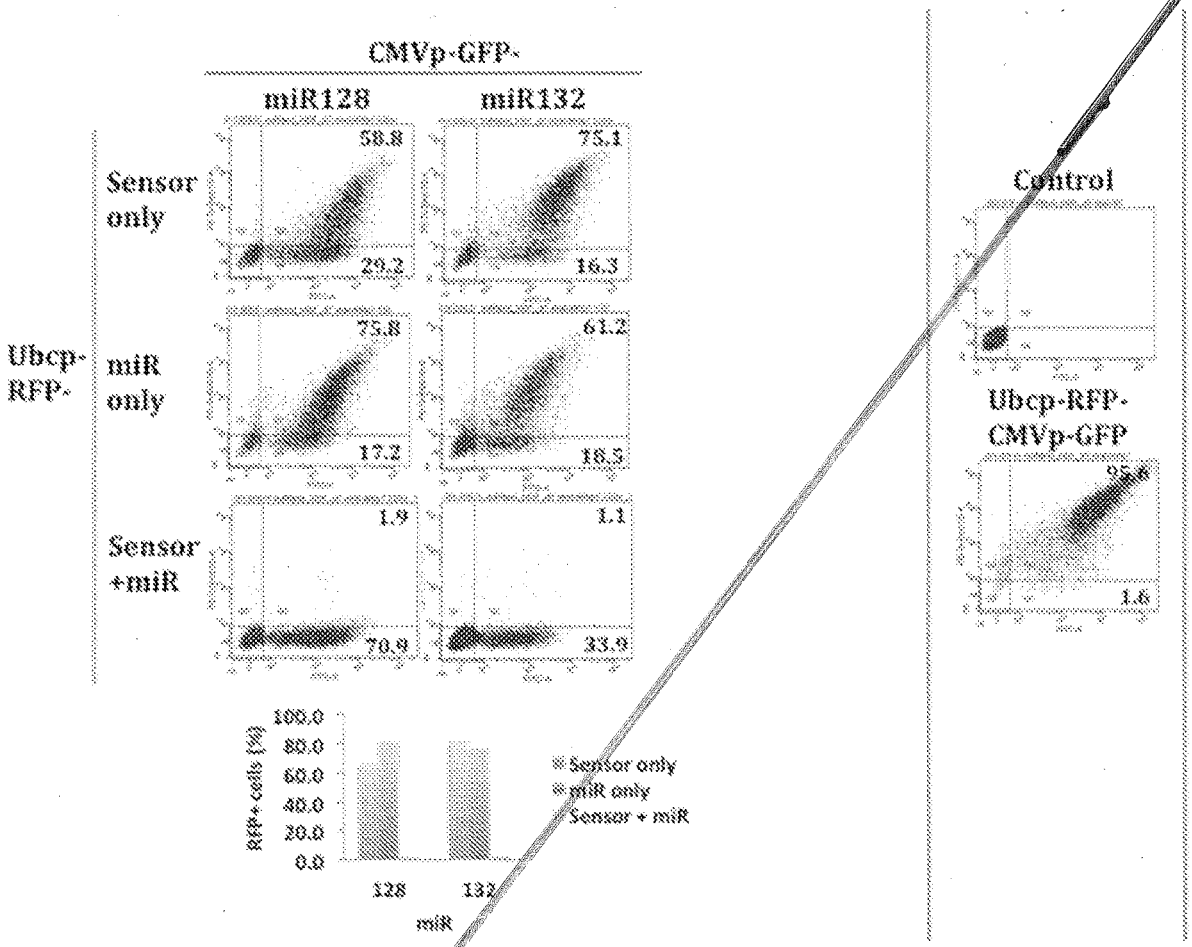
## Figure 33


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

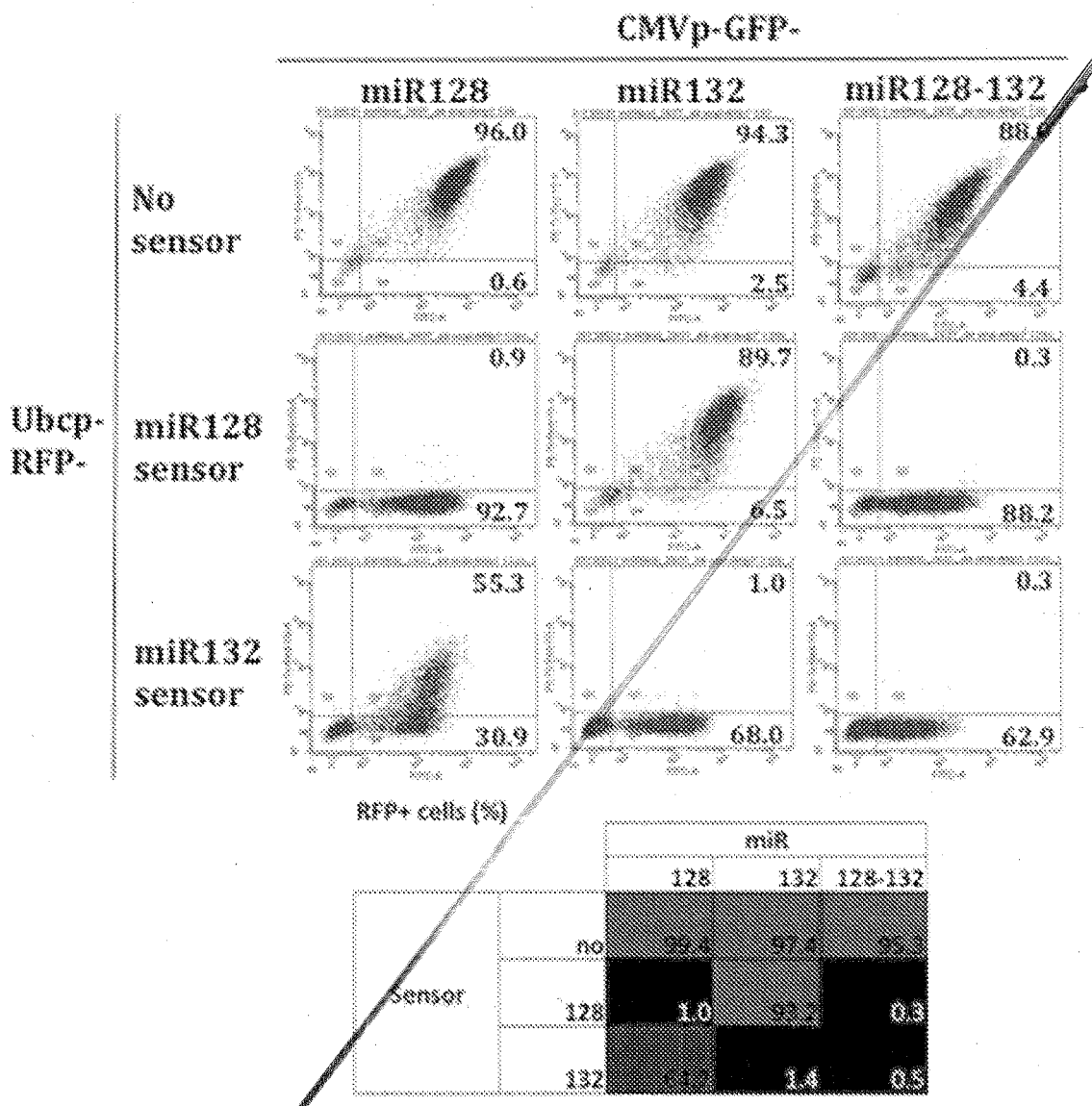
# Figure 34


CMVp-GFP-
miR124
miR128
miR132
Sensor only
miR only
Sensor +miR
Ubcp-RFP-
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
98.0
Control
Ubcp-RFP-CMVp-GFP
99.7
0.1
RFP+ cells [%]
100.0
80.0
60.0
40.0
20.0
0.0
124
128
132
miR
Sensor only
miR only
Sensor + miR

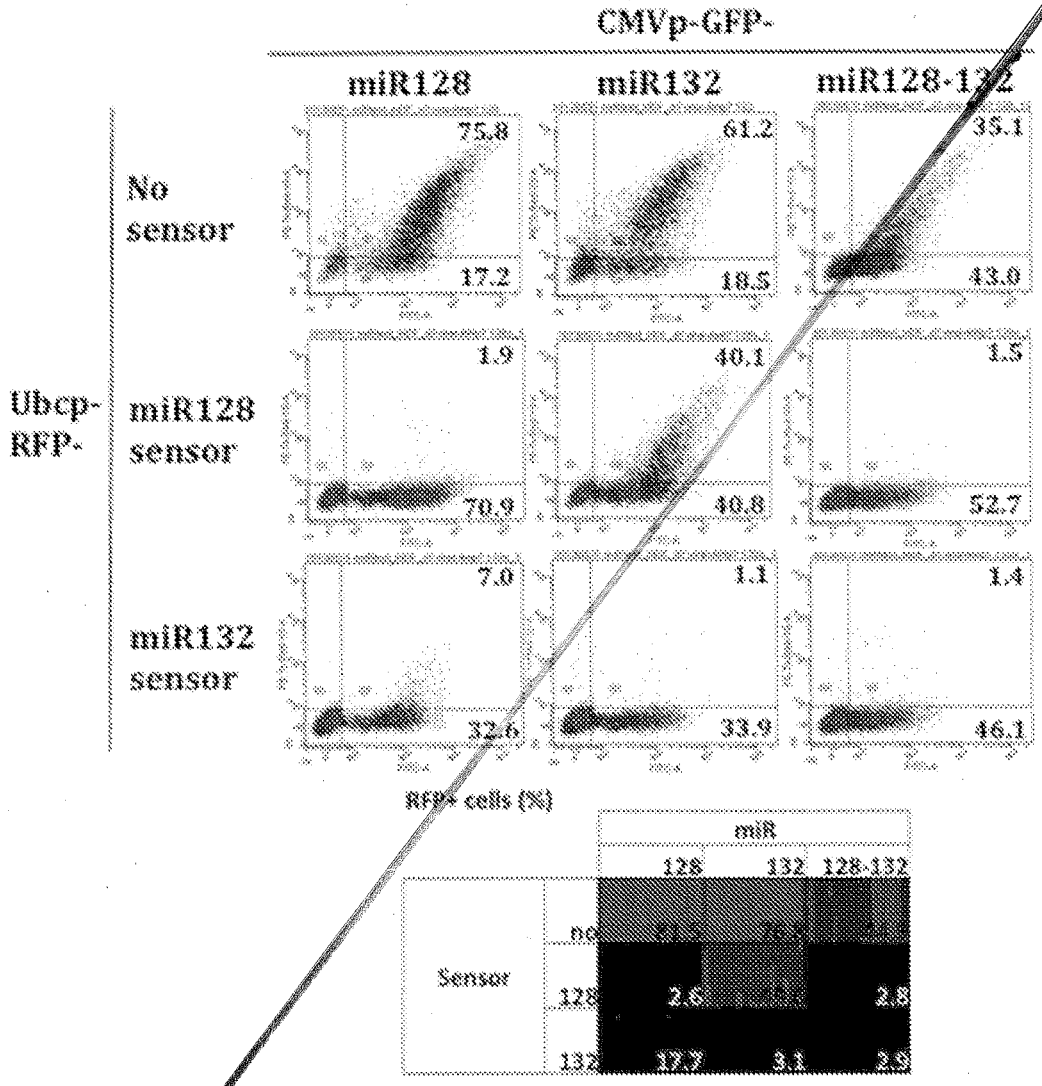
# Figure 35


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
16.5
1.9
70.9
1.1
33.9
Control
Ubcp-RFP-CMVp-GFP
95.8
1.6
RFP+ cells (%)
100.0
80.0
60.0
40.0
20.0
0.0
128
132
miR
Sensor only
miR only
Sensor + miR

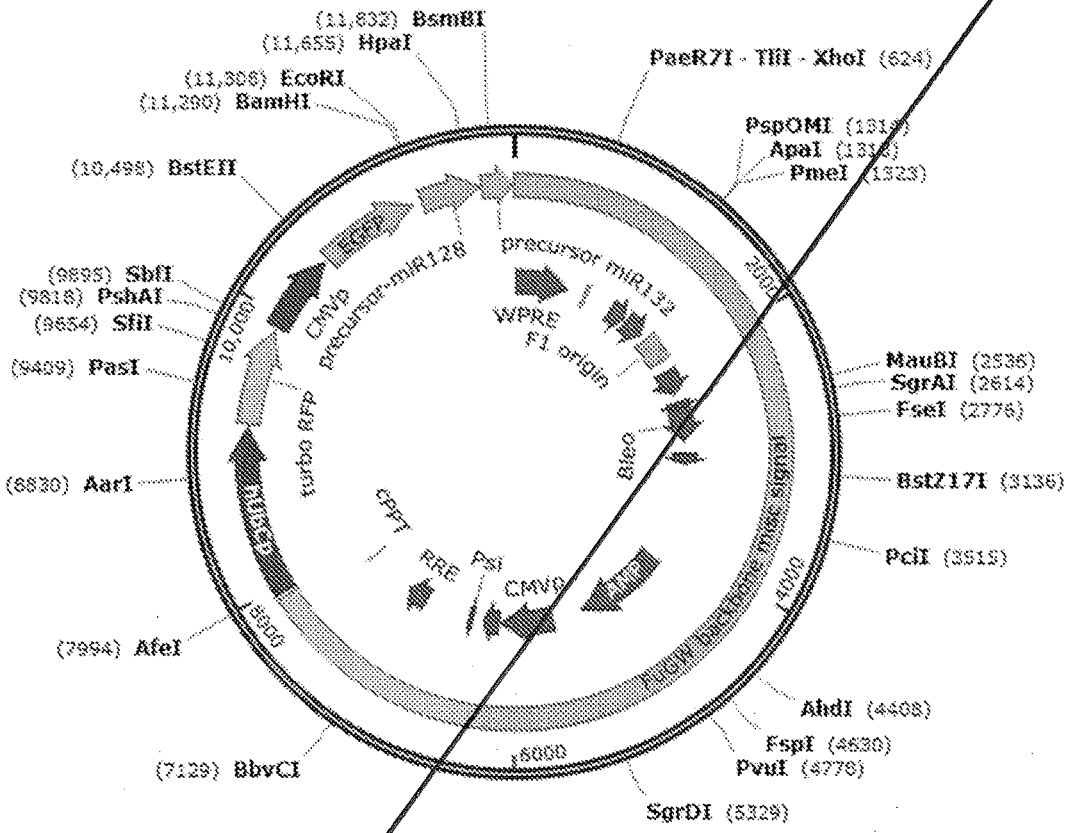
# Figure 36


CMVp-GFP-
miR128
miR132
miR128-132
Ubcp-RFP-
No sensor
miR128 sensor
miR132 sensor
96.0
0.6
94.3
2.5
88.6
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
99.4
97.4
95.3
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
12.7
3.1
2.8
2.5

# Figure 38


(11,632) BsmBI
(11,655) HpaI
(11,808) EcoRI
(11,290) BamHI
(10,498) BstEII
(9695) SbfI
(9818) PshAI
(9654) SfiI
(9409) PasI
(8830) AarI
(7994) AfeI
(7129) BbvCI
PaeR7I - TliI - XhoI (624)
PspOMI (1314)
ApaI (1316)
PmeI (1323)
MauBI (2536)
SgrAI (2614)
FseI (2776)
BstZ17I (3136)
PciI (3519)
AhdI (4408)
FspI (4630)
PvuI (4770)
SgrDI (5329)
precursor miR132
WPRE
f1 origin
BleO
CMVb
precursor-miR128
turbo RFP
cPPT
RRE
Psi
CMVd
EGFP
2000
4000
6000
8000
10,000