Figure 1

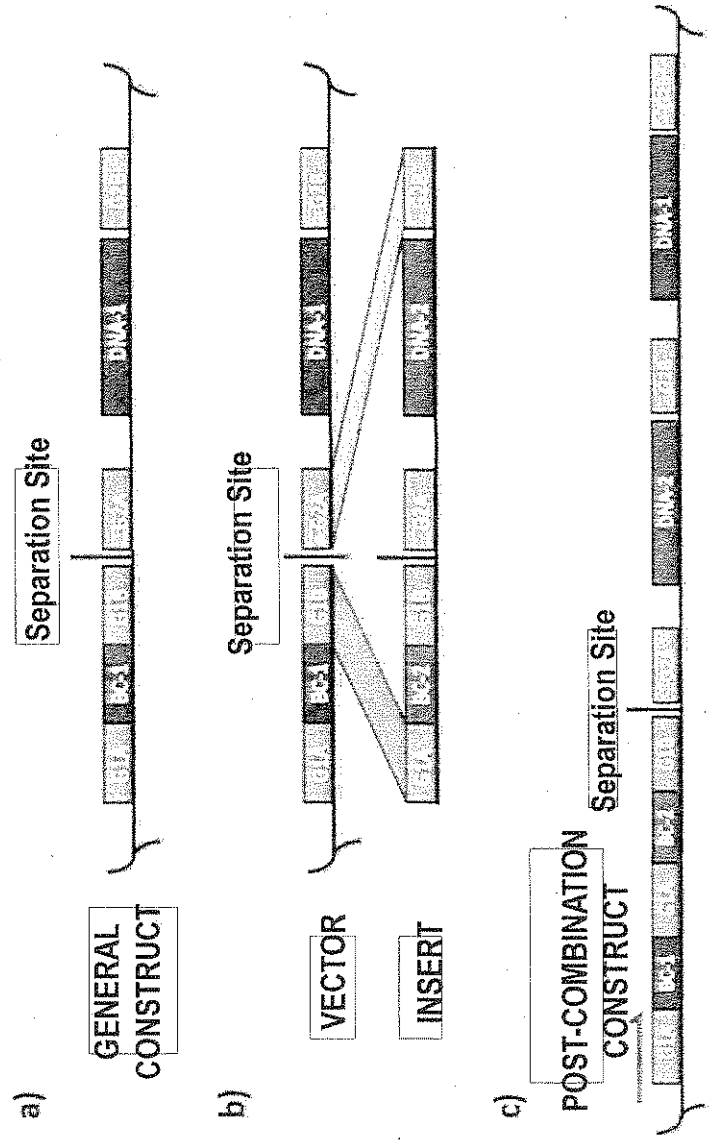
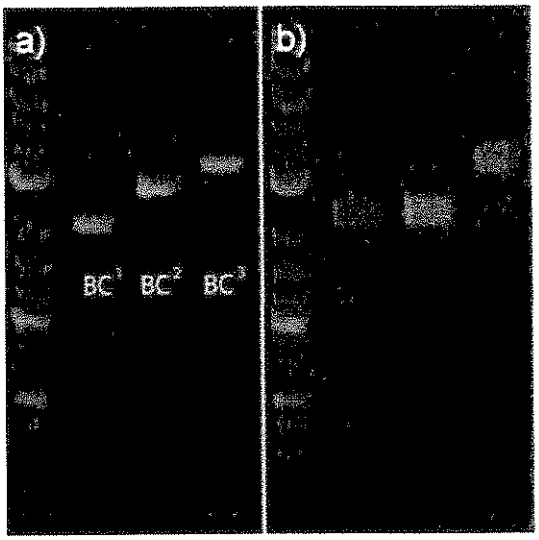

a)
Separation Site
GENERAL CONSTRUCT
b)
Separation Site
VECTOR
INSERT
c)
POST-COMBINATION CONSTRUCT
Separation Site

Figure 2



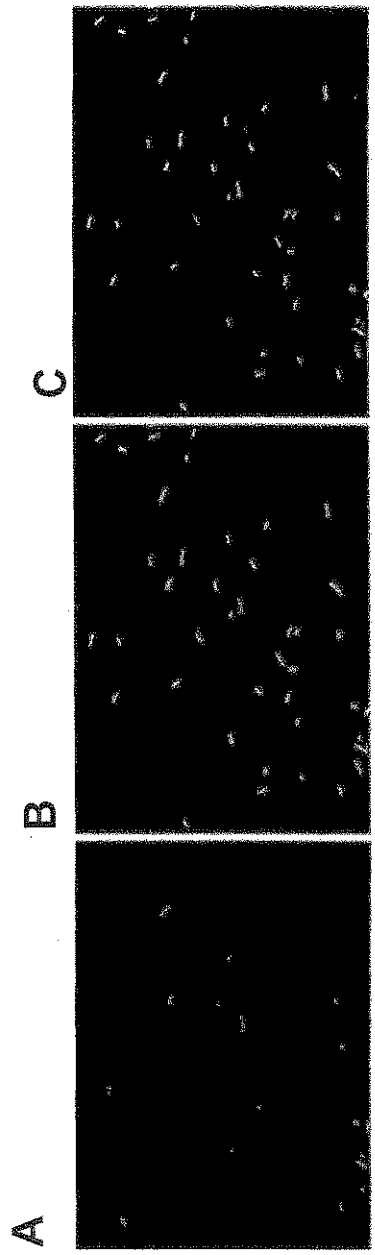


Figure 3

Figure 4

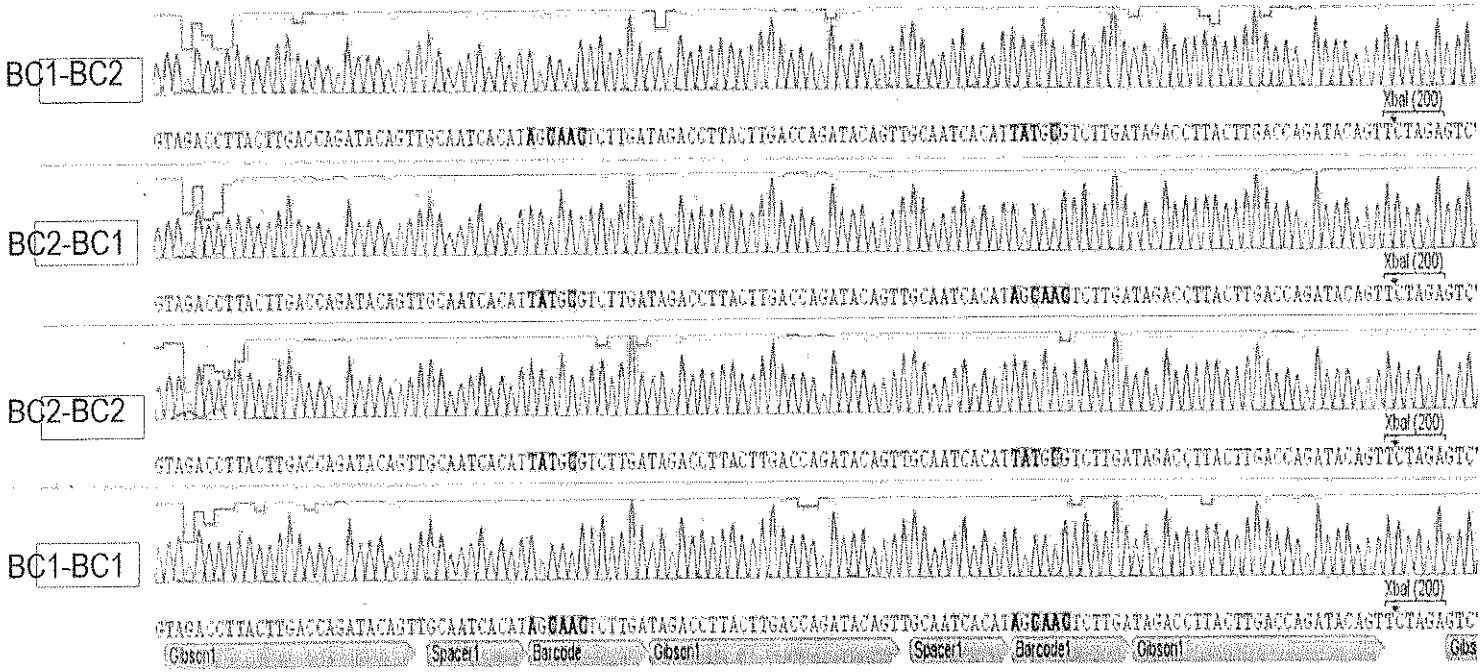

BC1-BC2
BC2-BC1
BC2-BC2
BC1-BC1
XbaI (200)
Gibson1
Spacer1
Barcode
Gibson1
Spacer1
Barcode1
Gibson1
Gibs

Figure 5

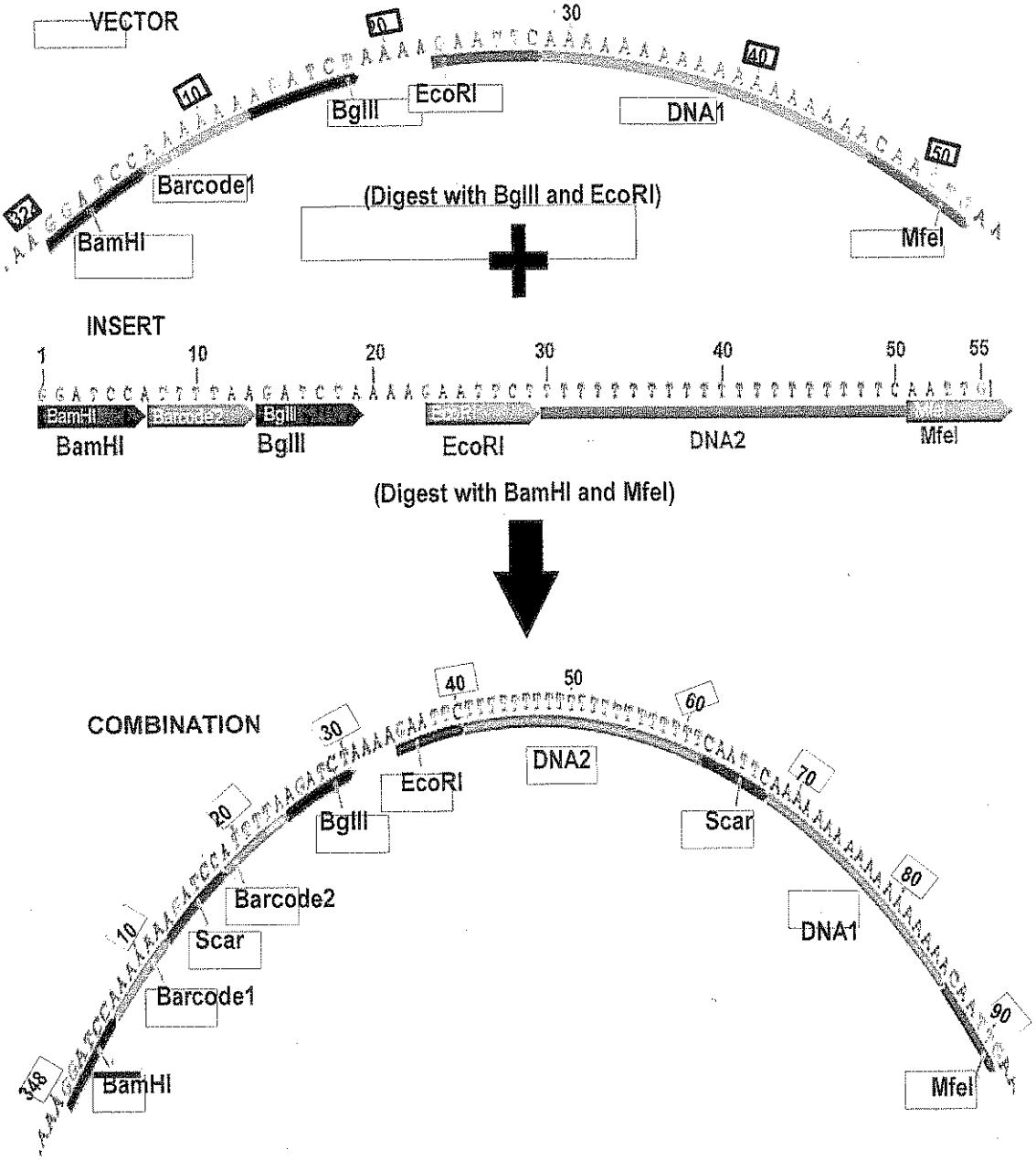
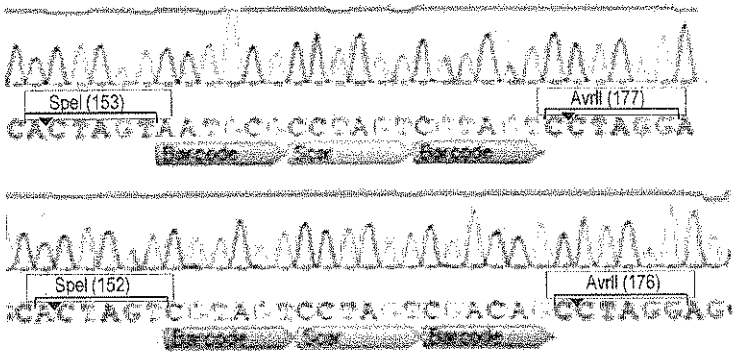
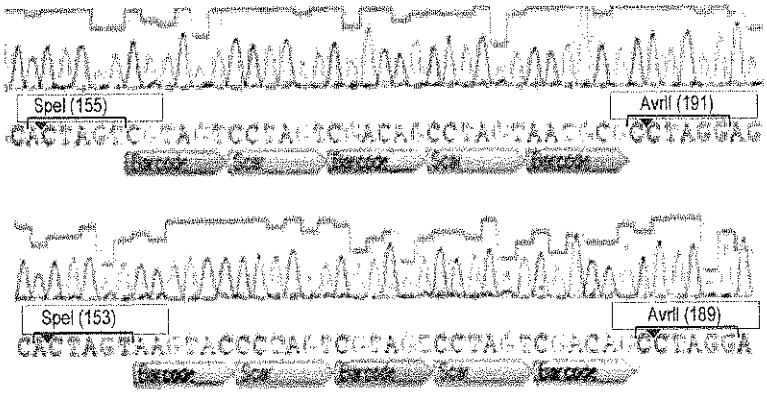

VECTOR
BglII
EcoRI
DNA1
Barcode1
BamHI
MfeI
(Digest with BglII and EcoRI)
INSERT
BamHI
BglII
EcoRI
DNA2
MfeI
(Digest with BamHI and MfeI)
COMBINATION
BglII
EcoRI
DNA2
Scar
Barcode2
Scar
Barcode1
DNA1
BamHI
MfeI

Figure 6

a) 2x Combination



3x Combination



4x Combination

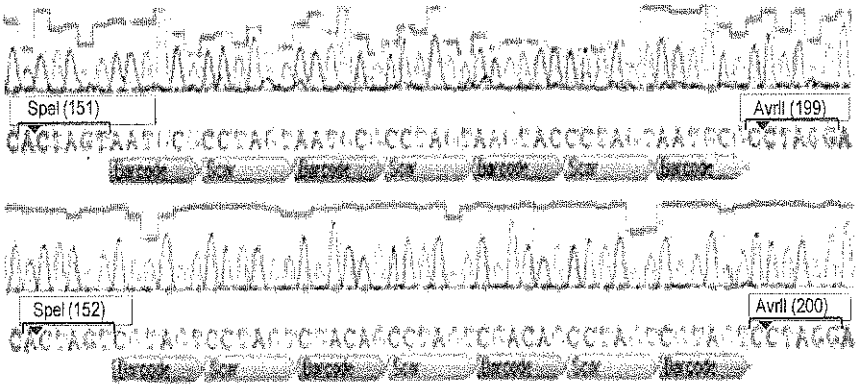


Figure 6

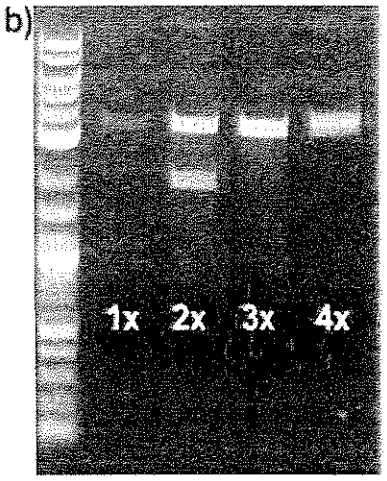


Figure 7

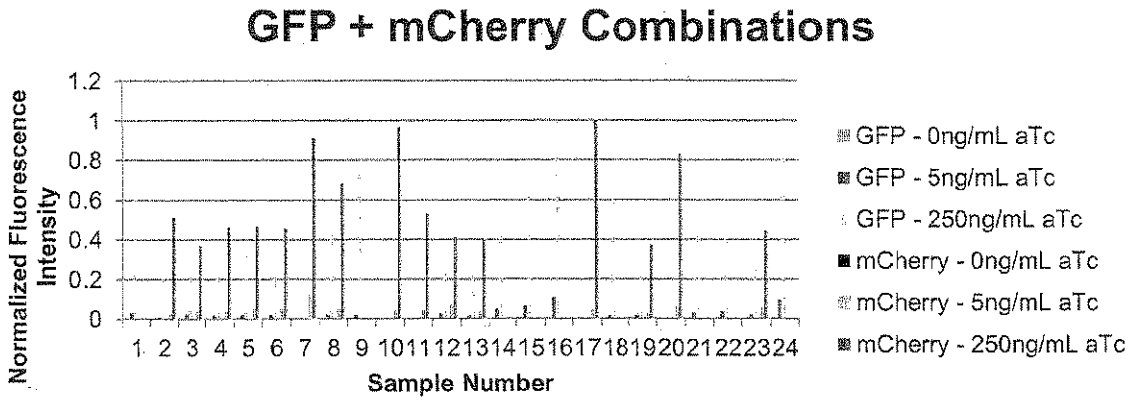


Figure 8

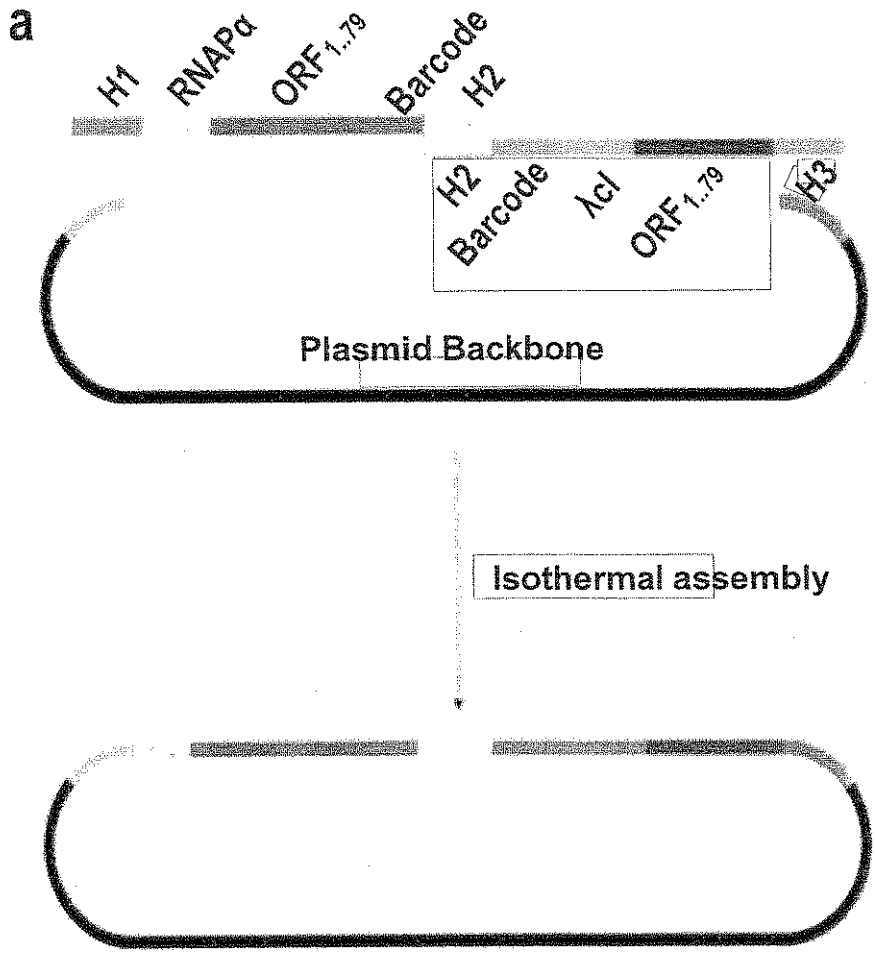
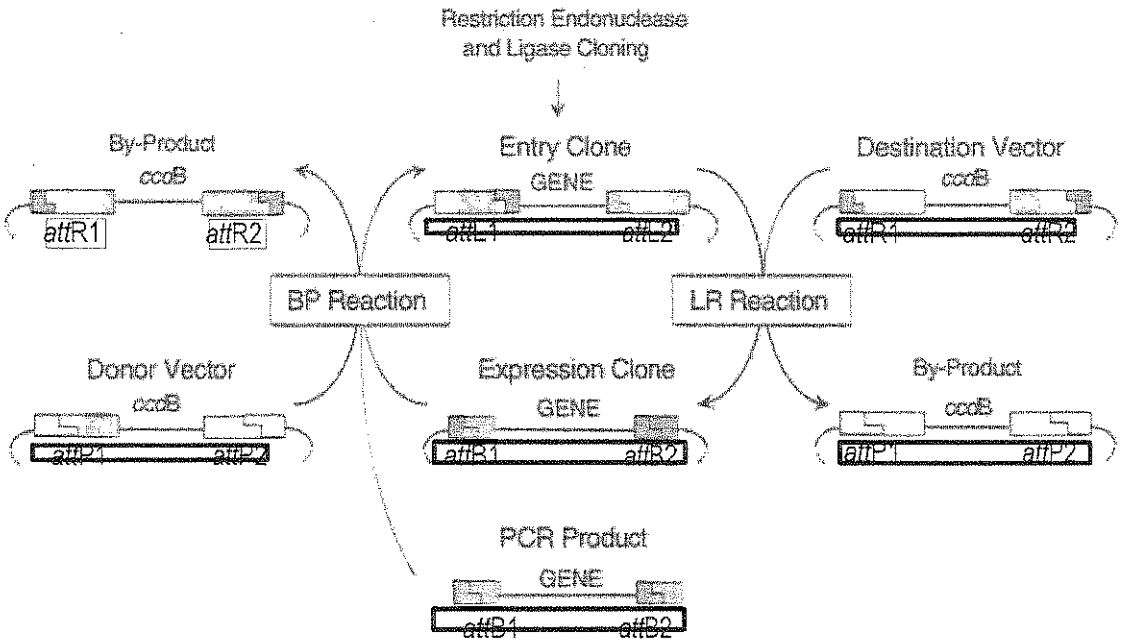

a
H1
RNAPα
ORF1..79
Barcode
H2
H2
Barcode
λcI
ORF1..79
H3
Plasmid Backbone
Isothermal assembly

Figure 9



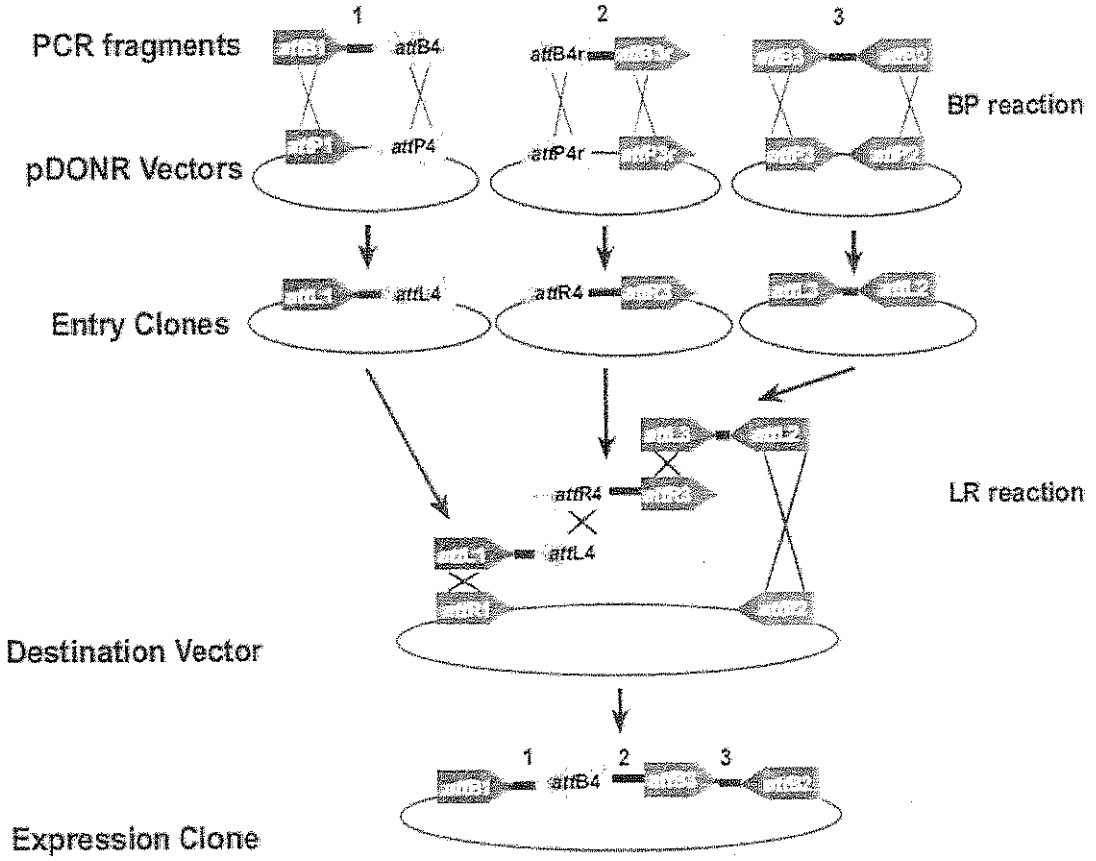
Key:

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

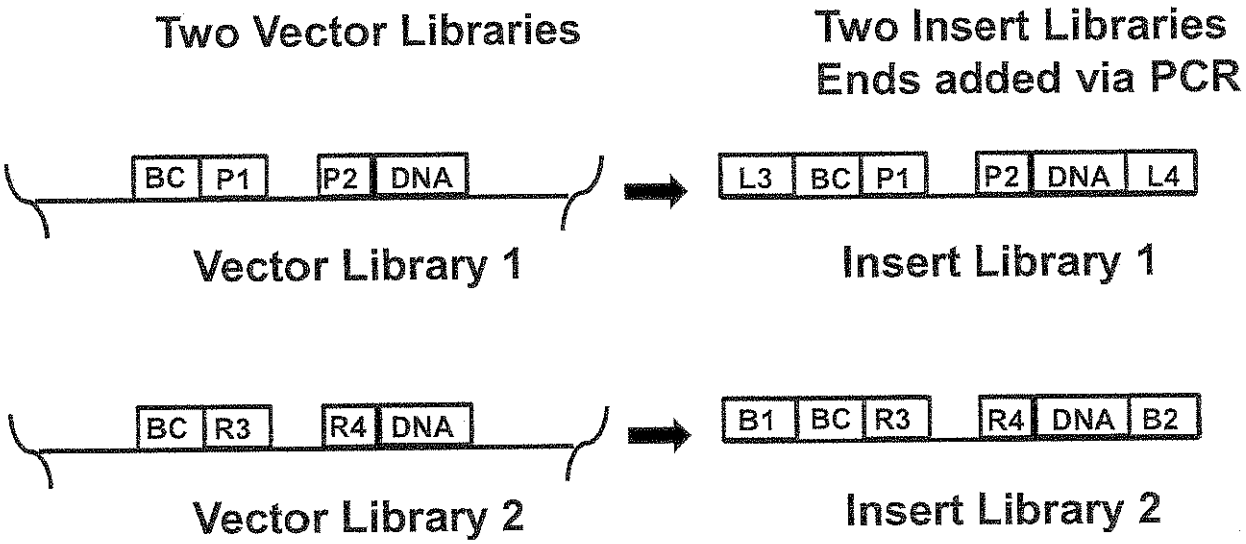

Two Vector Libraries
Two Insert Libraries
Ends added via PCR
BC
P1
P2
DNA
L3
BC
P1
P2
DNA
L4
Vector Library 1
Insert Library 1
BC
R3
R4
DNA
B1
BC
R3
R4
DNA
B2
Vector Library 2
Insert Library 2

Figure 12

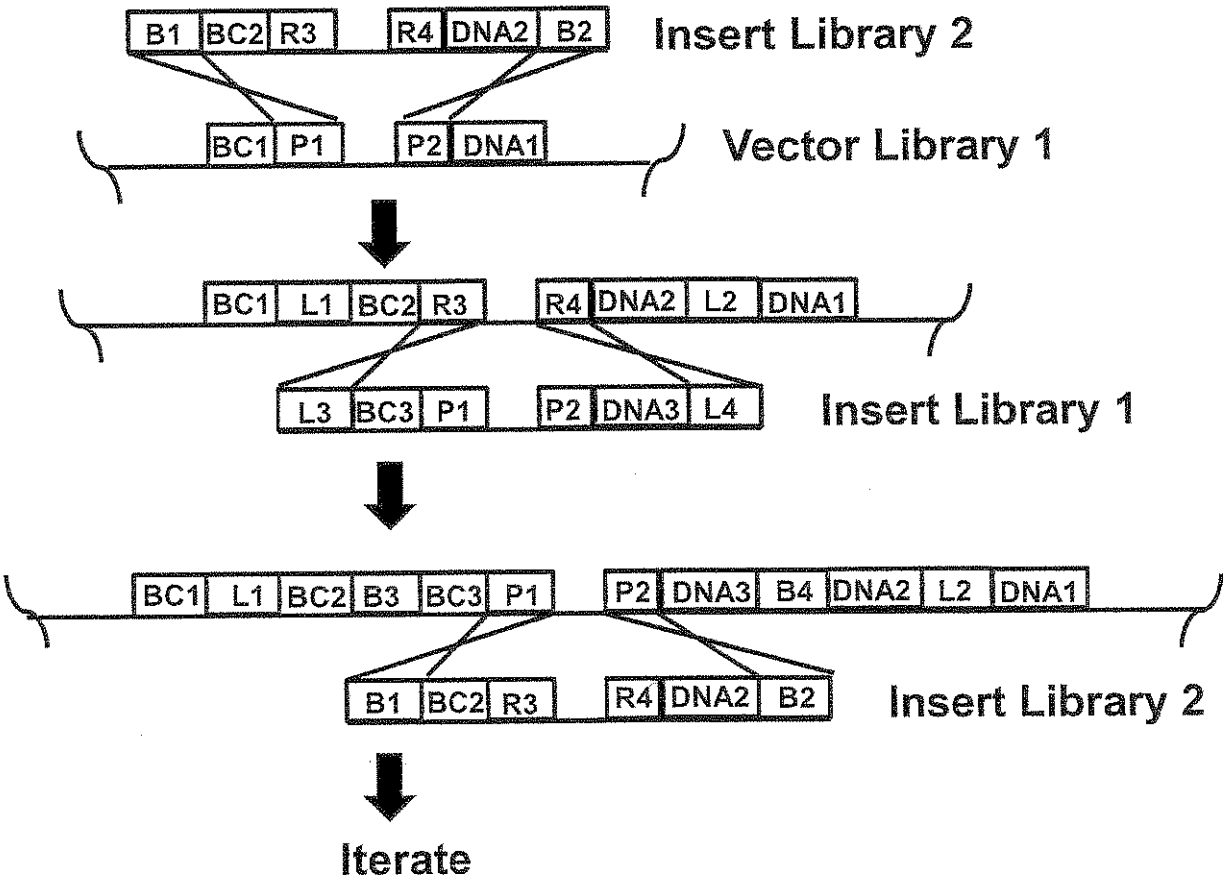

B1
BC2
R3
R4
DNA2
B2
Insert Library 2
BC1
P1
P2
DNA1
Vector Library 1
BC1
L1
BC2
R3
R4
DNA2
L2
DNA1
L3
BC3
P1
P2
DNA3
L4
Insert Library 1
BC1
L1
BC2
B3
BC3
P1
P2
DNA3
B4
DNA2
L2
DNA1
B1
BC2
R3
R4
DNA2
B2
Insert Library 2
Iterate

Figure 13

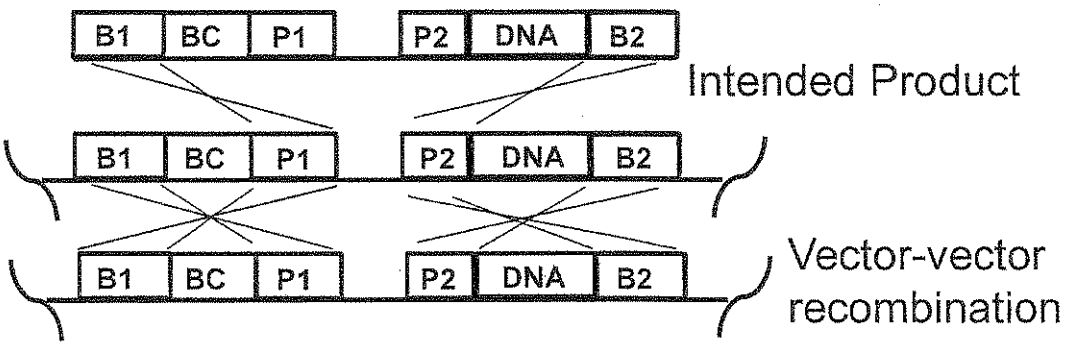

B1
BC
P1
P2
DNA
B2
Intended Product
B1
BC
P1
P2
DNA
B2
B1
BC
P1
P2
DNA
B2
Vector-vector
recombination

Figure 14

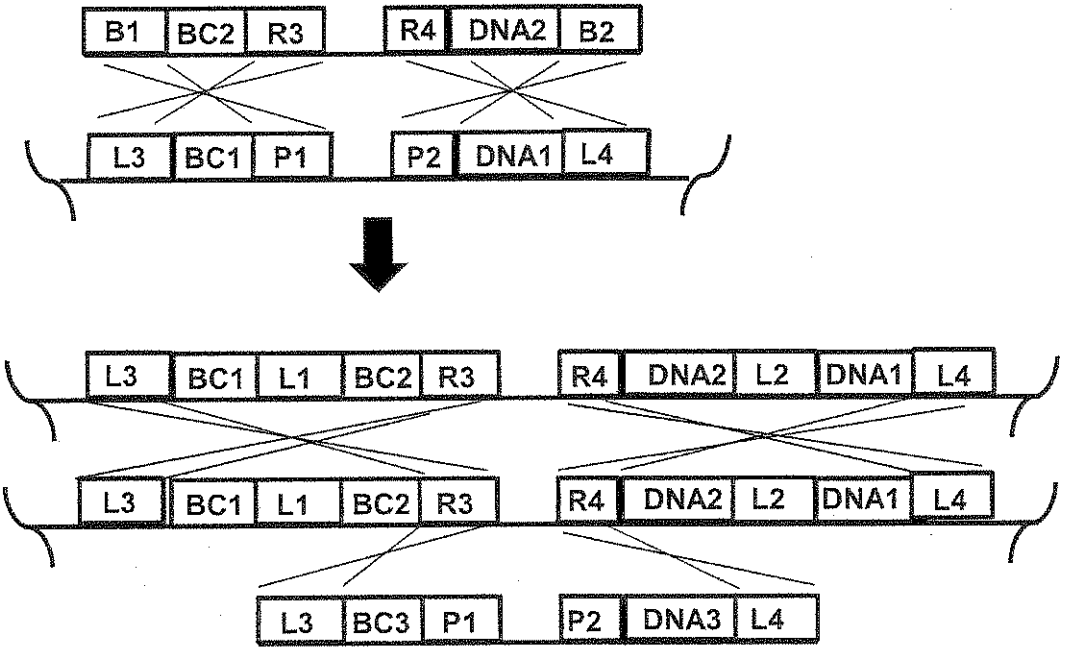

B1
BC2
R3
R4
DNA2
B2
L3
BC1
P1
P2
DNA1
L4
L3
BC1
L1
BC2
R3
R4
DNA2
L2
DNA1
L4
L3
BC1
L1
BC2
R3
R4
DNA2
L2
DNA1
L4
L3
BC3
P1
P2
DNA3
L4

Figure 15

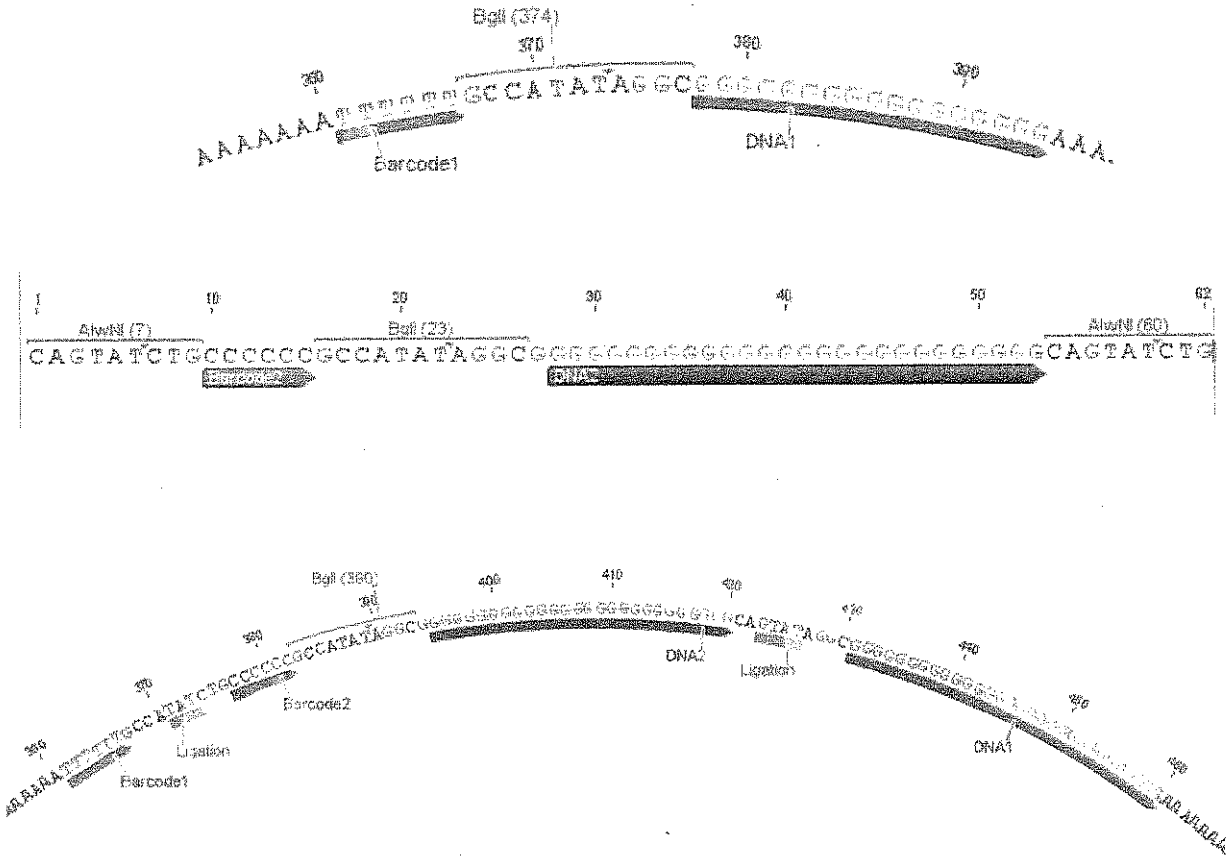

BglI (374)
370
380
390
Barcode1
DNA1
AlwNI (7)
BglI (23)
AlwNI (60)
BglI (389)
400
410
420
DNA2
Ligation
Barcode2
Ligation
Barcode1
DNA1

Figure 16

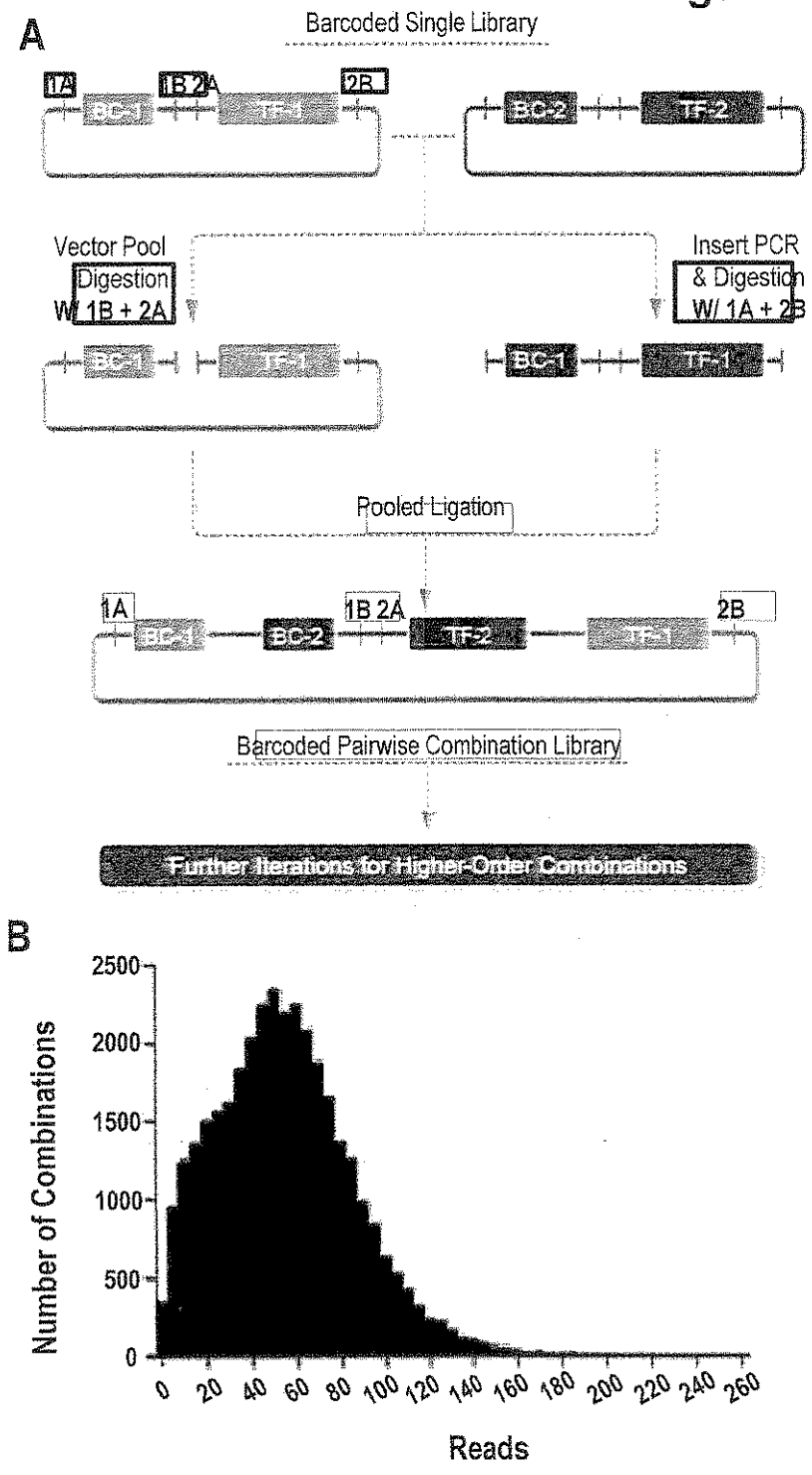

A
Barcoded Single Library
1A
1B 2A
2B
BC-1
TF-1
BC-2
TF-2
Vector Pool
Digestion
W/ 1B + 2A
Insert PCR
& Digestion
W/ 1A + 2B
Pooled Ligation
Barcoded Pairwise Combination Library
Further Iterations for Higher-Order Combinations
B
Number of Combinations
2500
2000
1500
1000
500
0
0 20 40 60 80 100 120 140 160 180 200 220 240 260
Reads

Figure 16

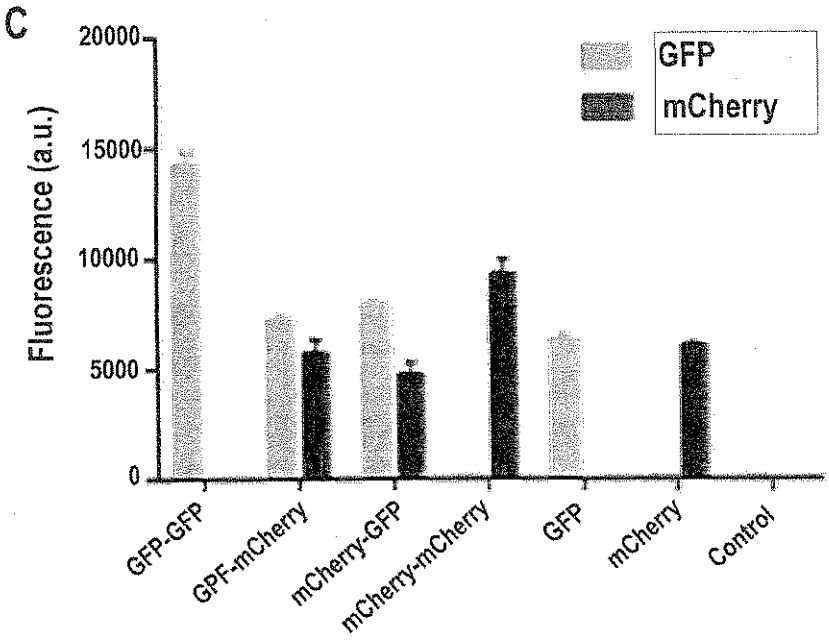


Figure 17

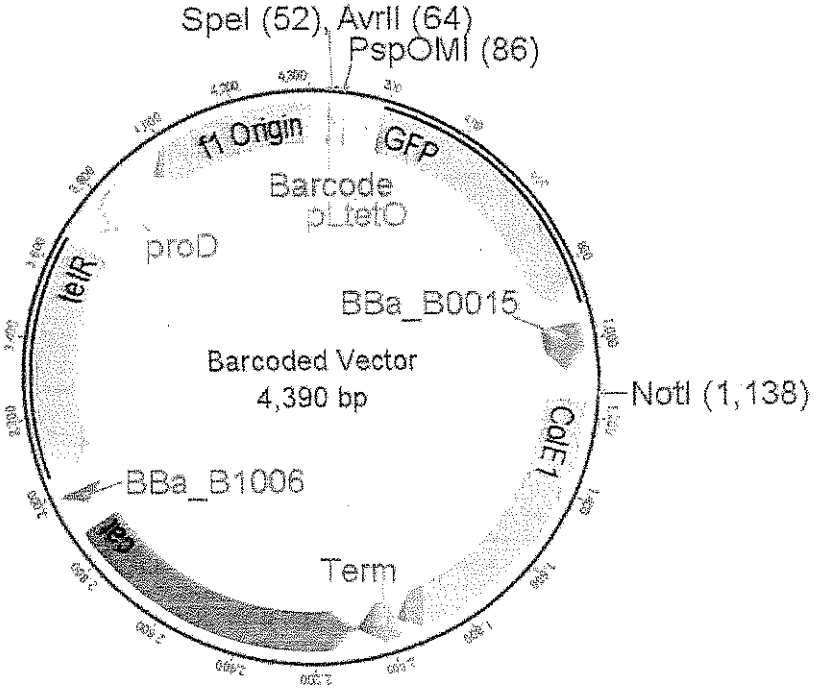


Figure 18

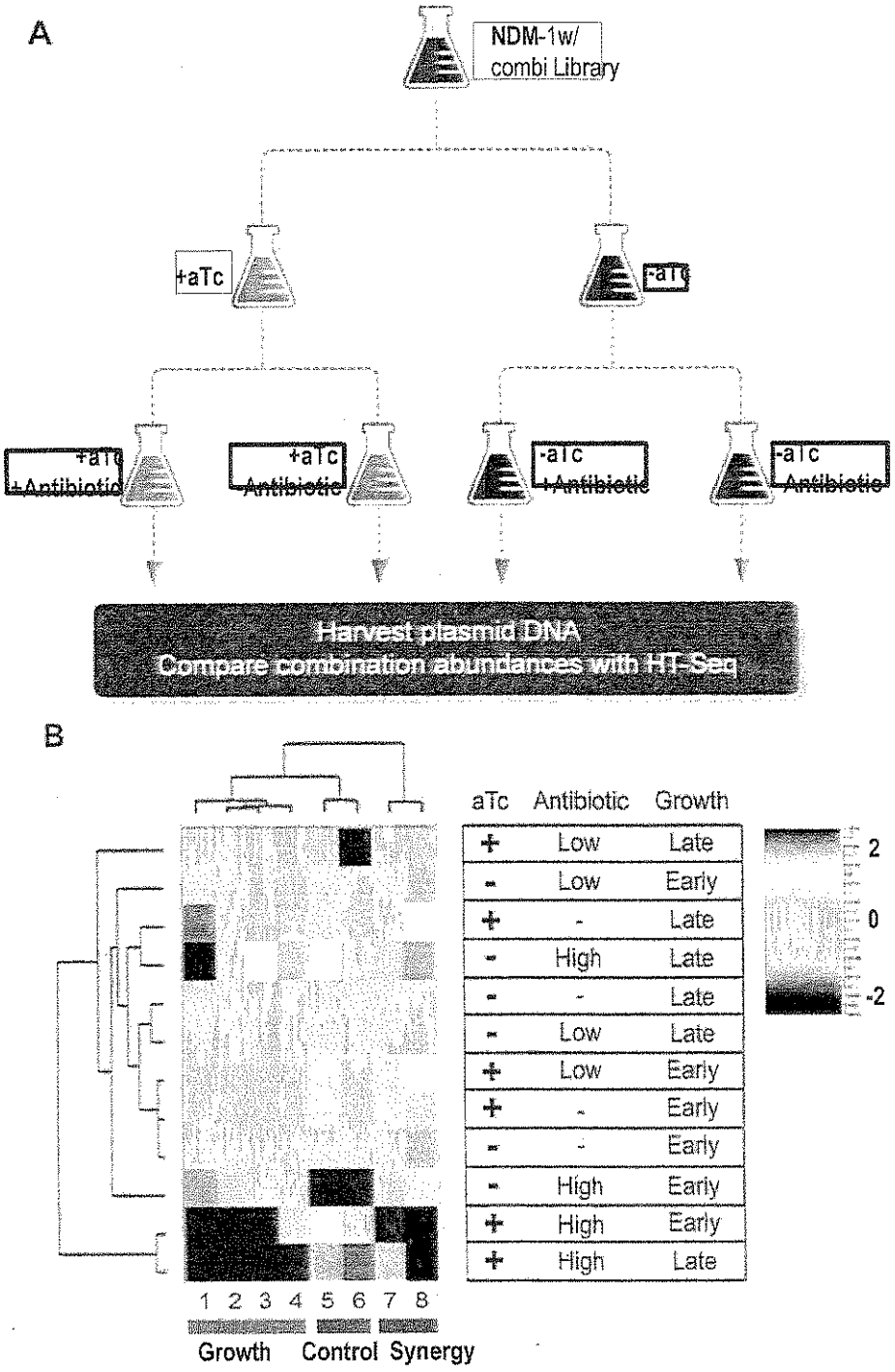

A
NDM-1w/
combi Library
+aTc
-aTc
+aTc
+Antibiotic
+aTc
Antibiotic
-aTc
+Antibiotic
-aTc
Antibiotic
Harvest plasmid DNA
Compare combination abundances with HT-Seq
B
aTc Antibiotic Growth
+ Low Late
- Low Early
+ - Late
- High Late
- - Late
- Low Late
+ Low Early
+ - Early
- - Early
- High Early
+ High Early
+ High Late
2
0
-2
1 2 3 4 5 6 7 8
Growth Control Synergy

Figure 19

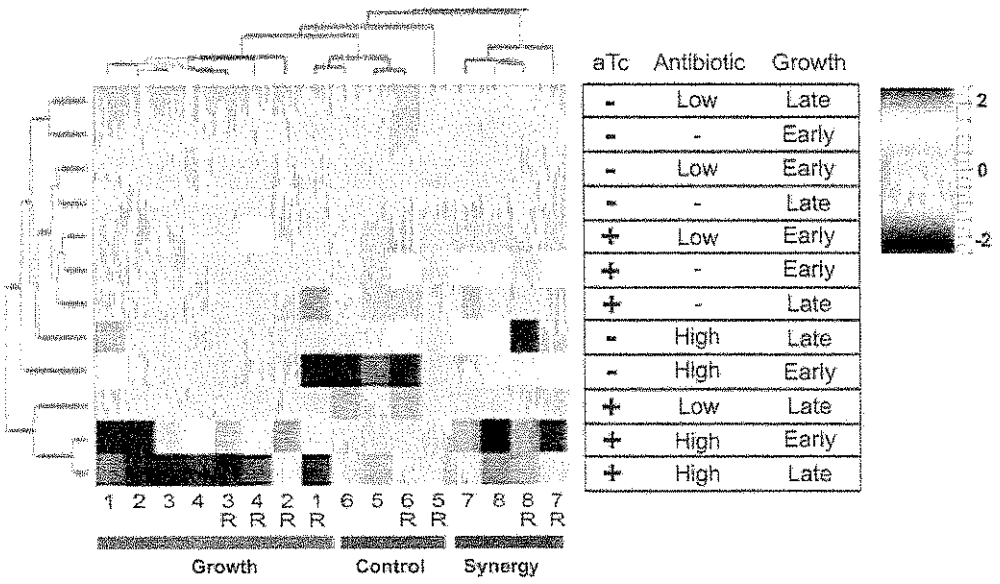

aTc
Antibiotic
Growth
- Low Late
- - Early
- Low Early
- - Late
+ Low Early
+ - Early
+ - Late
- High Late
- High Early
+ Low Late
+ High Early
+ High Late
2
0
-2
1 2 3 4 3R 4R 2R 1R 6 5 6R 5R 7 8 8R 7R
Growth
Control
Synergy

Figure 20

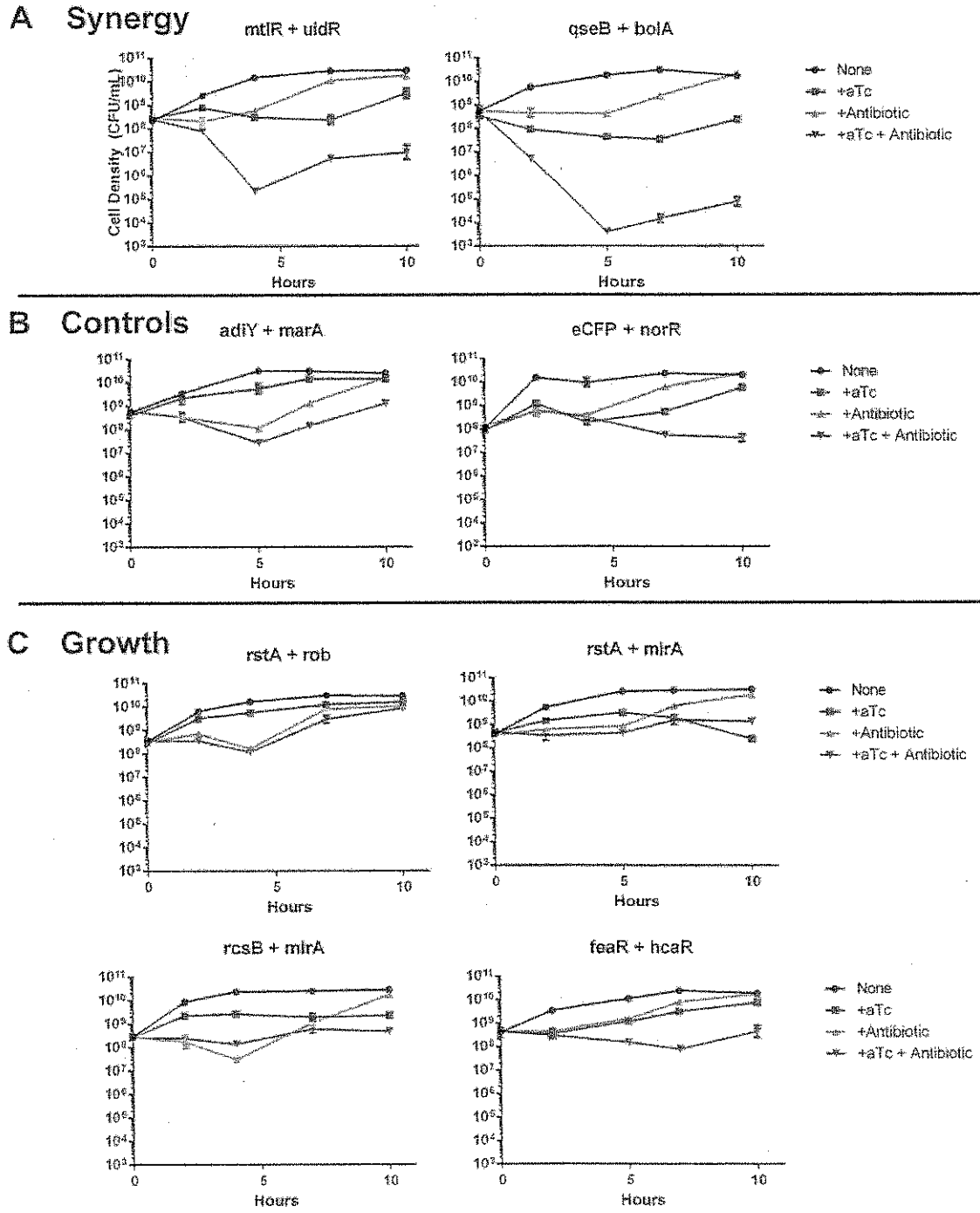

A Synergy
mtlR + uidR
qseB + bolA
Cell Density (CFU/mL)
Hours
None
+aTc
+Antibiotic
+aTc + Antibiotic
B Controls
adiY + marA
eCFP + norR
C Growth
rstA + rob
rstA + mlrA
rcsB + mlrA
feaR + hcaR

Figure 21

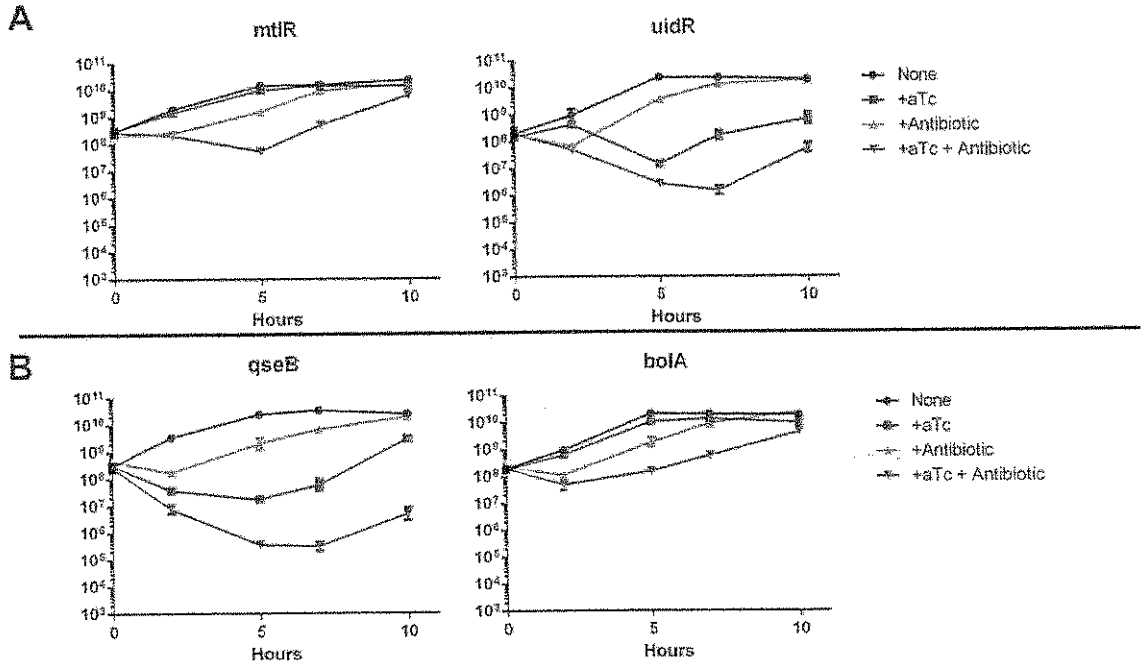

A
mtlR
uidR
None
+aTc
+Antibiotic
+aTc + Antibiotic
Hours
B
qseB
bolA
None
+aTc
+Antibiotic
+aTc + Antibiotic
Hours

Figure 22

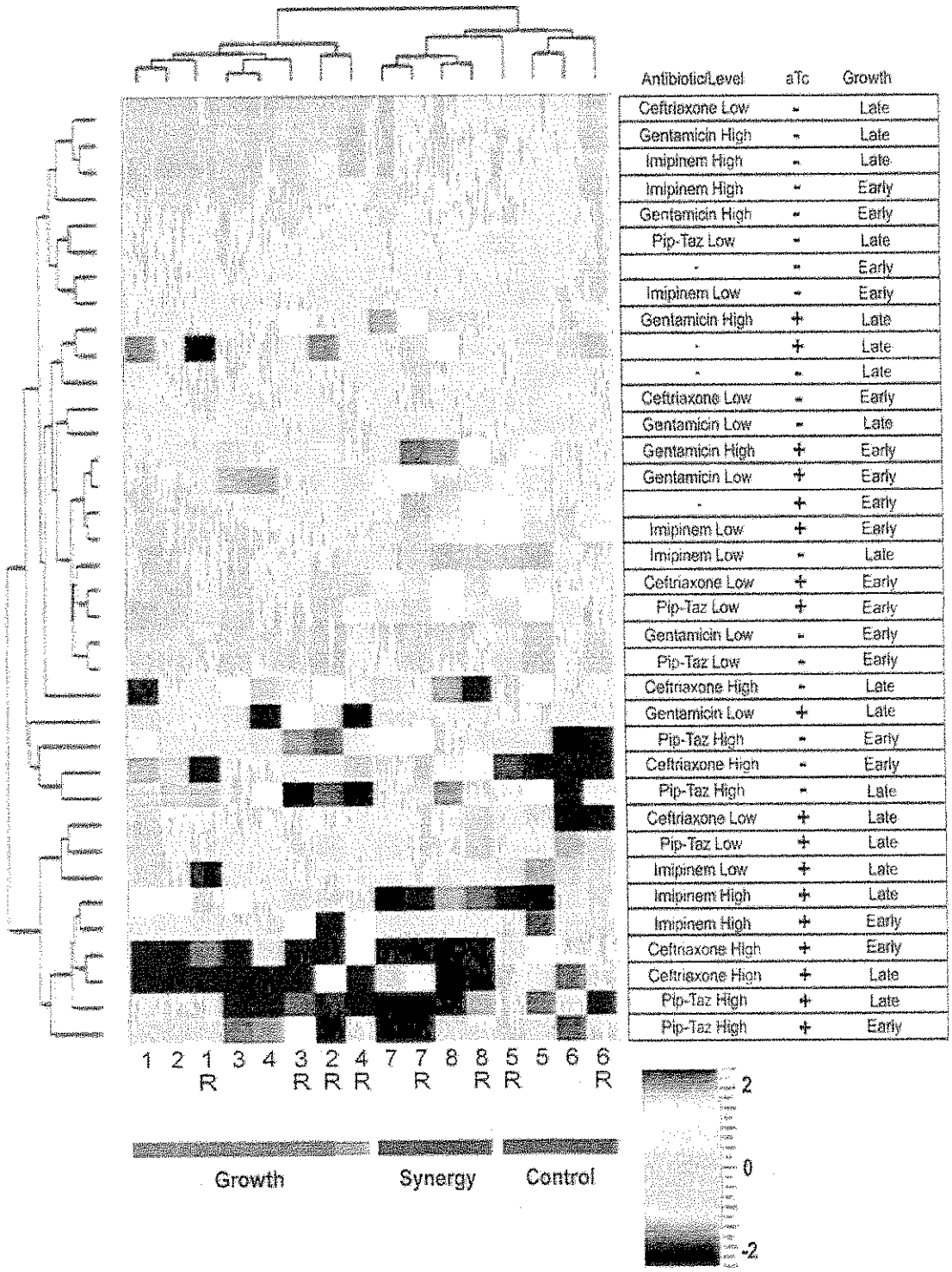

AntibioticLevel aTc Growth
Ceftriaxone Low - Late
Gentamicin High - Late
Imipinem High - Late
Imipinem High - Early
Gentamicin High - Early
Pip-Taz Low - Late
- - Early
Imipinem Low - Early
Gentamicin High + Late
- + Late
- - Late
Ceftriaxone Low - Early
Gentamicin Low - Late
Gentamicin High + Early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

Figure 23

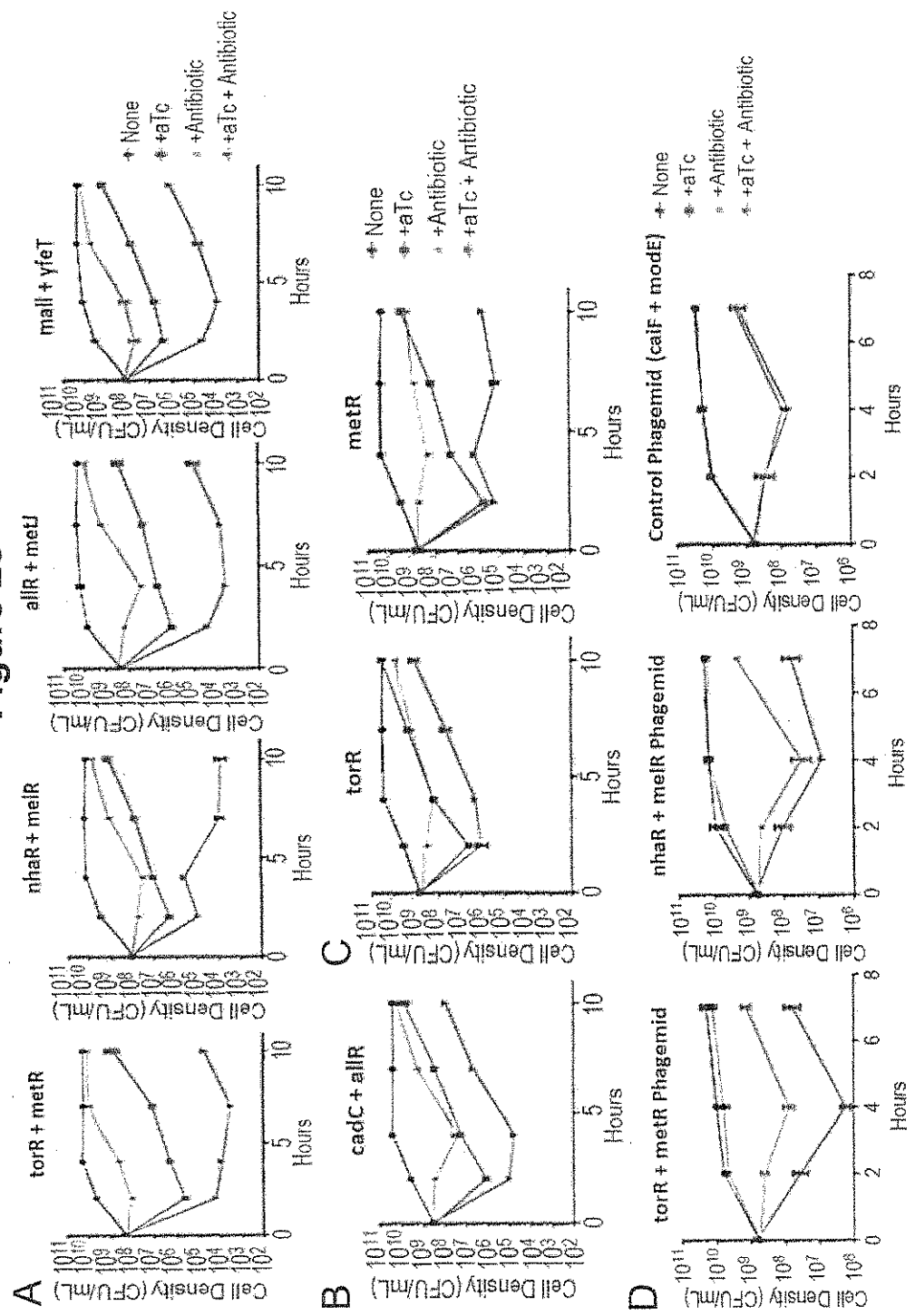

A
torR + metR
nhaR + melR
allR + metJ
mall + yfeT
B
cadC + allR
C
torR
metR
D
torR + metR Phagemid
nhaR + melR Phagemid
Control Phagemid (caiF + modE)
Cell Density (CFU/mL)
Hours
None
+aTc
+Antibiotic
+aTc + Antibiotic

Figure 24

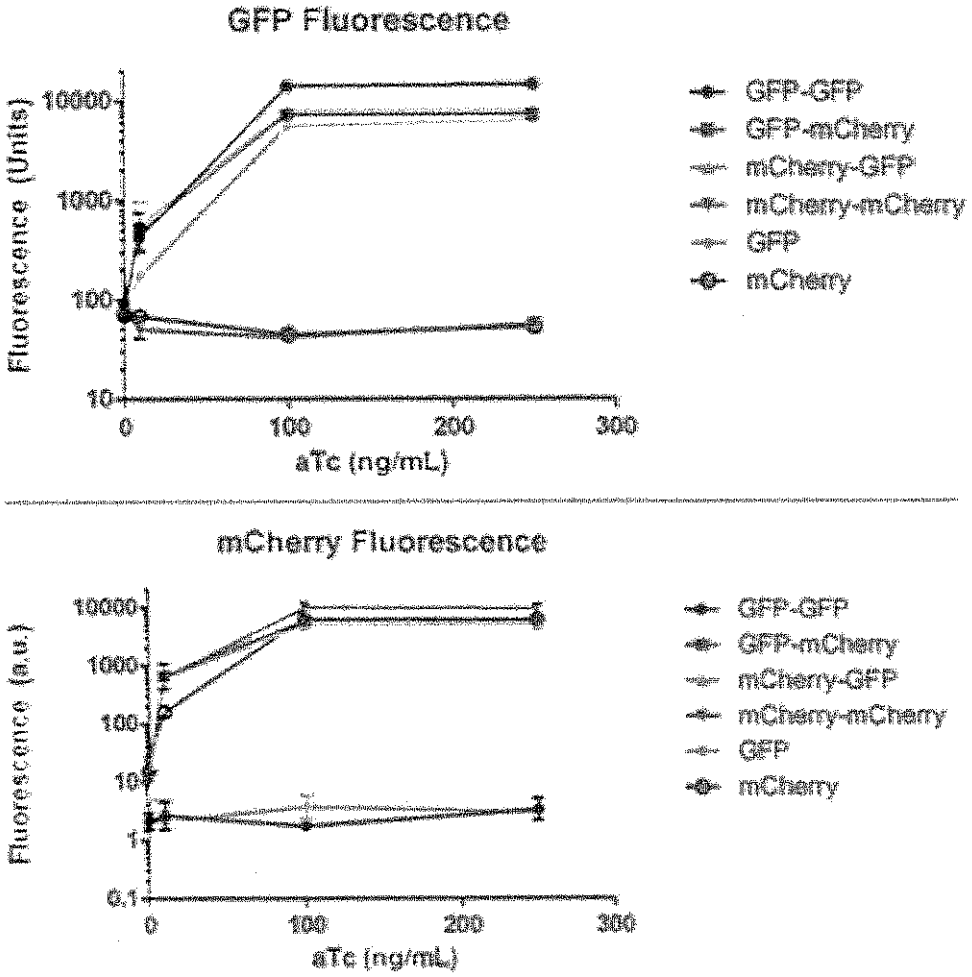

GFP Fluorescence
Fluorescence (Units)
10000
1000
100
10
0
100
200
300
aTc (ng/mL)
GFP-GFP
GFP-mCherry
mCherry-GFP
mCherry-mCherry
GFP
mCherry
mCherry Fluorescence
Fluorescence (a.u.)
10000
1000
100
10
1
0,1
0
100
200
300
aTc (ng/mL)
GFP-GFP
GFP-mCherry
mCherry-GFP
mCherry-mCherry
GFP
mCherry

Figure 25

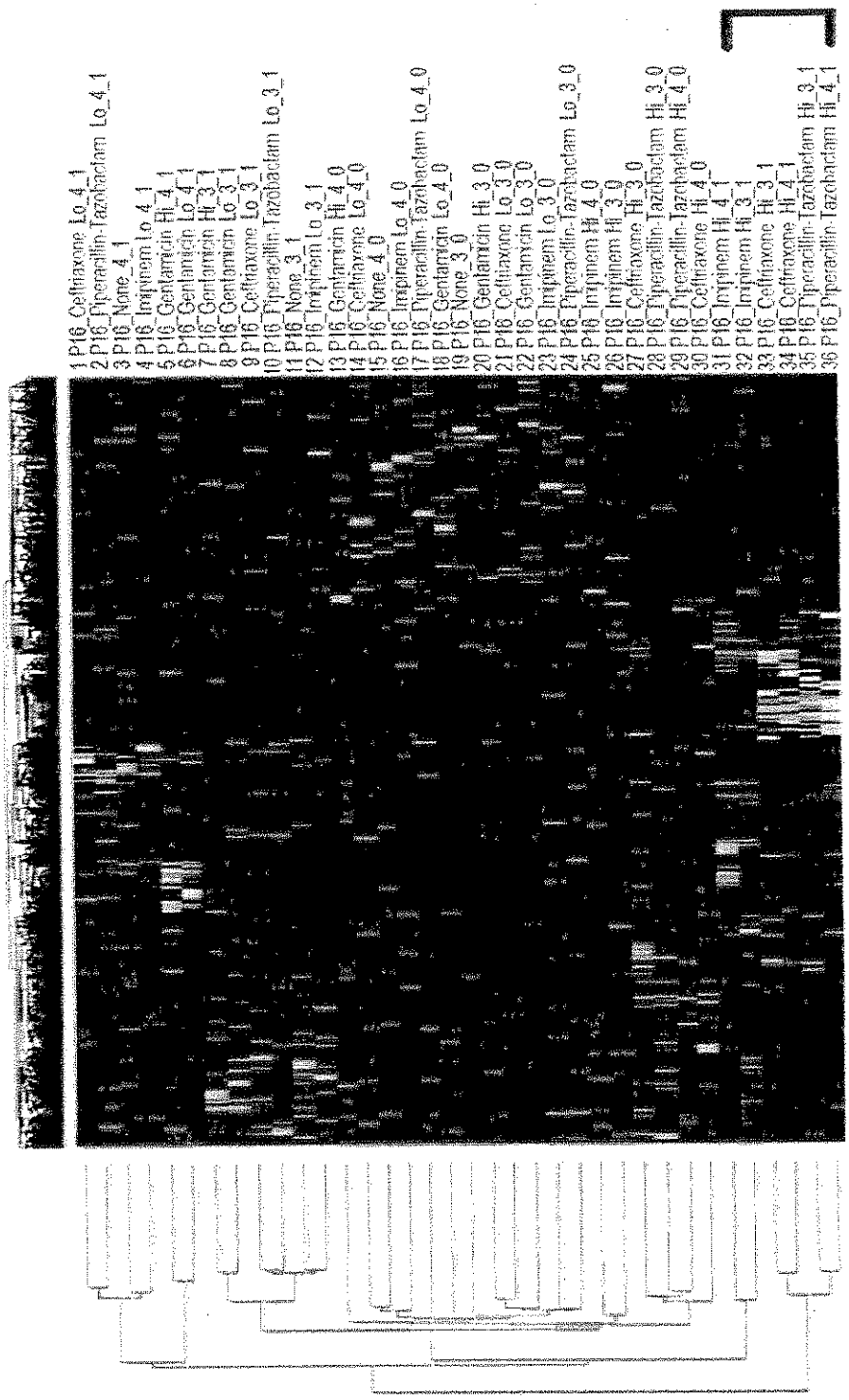

1 P16_Ceftriaxone Lo_4_1
2 P16_Piperacillin-Tazobactam Lo_4_1
3 P16_None_4_1
4 P16_Imipinem Lo_4_1
5 P16_Gentamicin Hi_4_1
6 P16_Gentamicin Lo_4_1
7 P16_Gentamicin Hi_3_1
8 P16_Gentamicin Lo_3_1
9 P16_Ceftriaxone Lo_3_1
10 P16_Piperacillin-Tazobactam Lo_3_1
11 P16_None_3_1
12 P16_Imipinem Lo_3_1
13 P16_Gentamicin 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

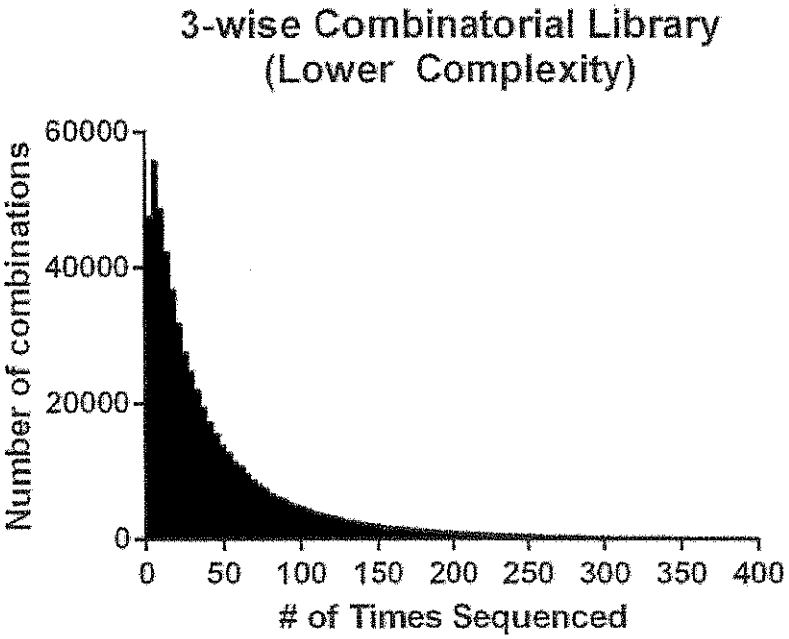


Figure 27

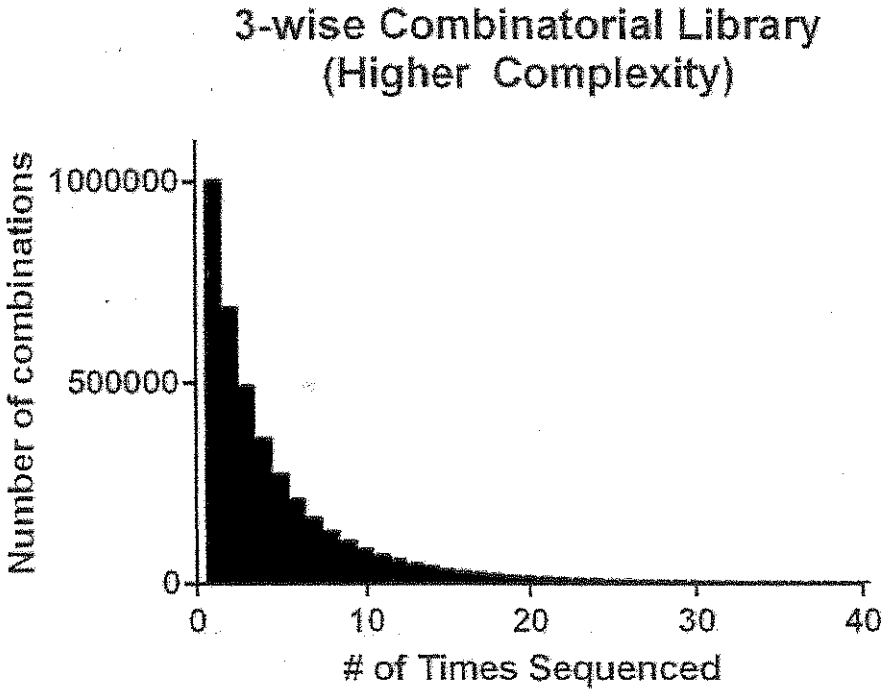
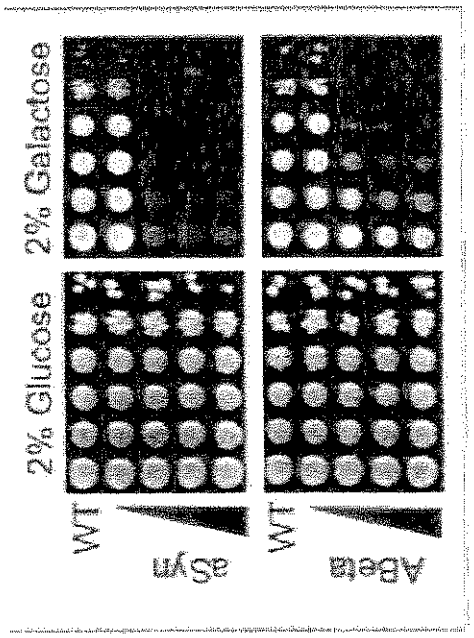


Figure 28


2% Glucose
2% Galactose
WT
aSyn
WT
Abeta

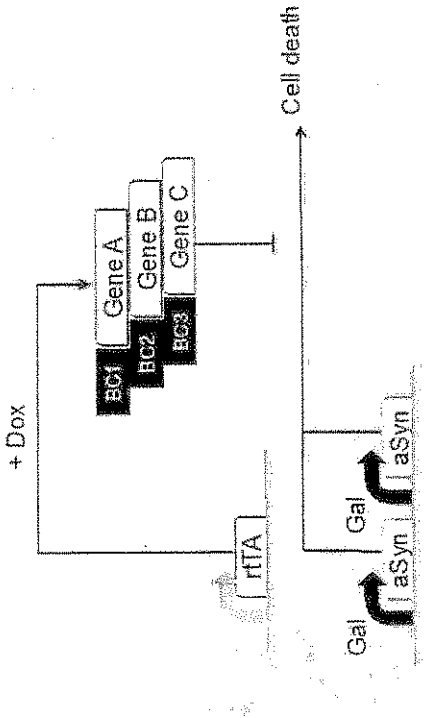

+ Dox
Gene A
Gene B
Gene C
BC1
BC2
BC3
rtTA
Gal
aSyn
Gal
aSyn
Cell death

Figure 29

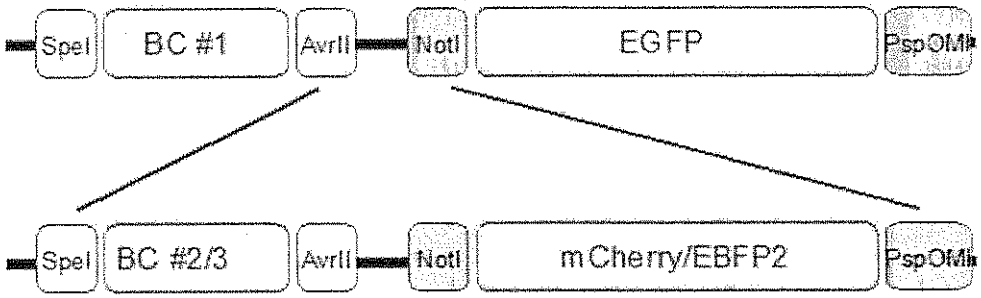


Figure 30

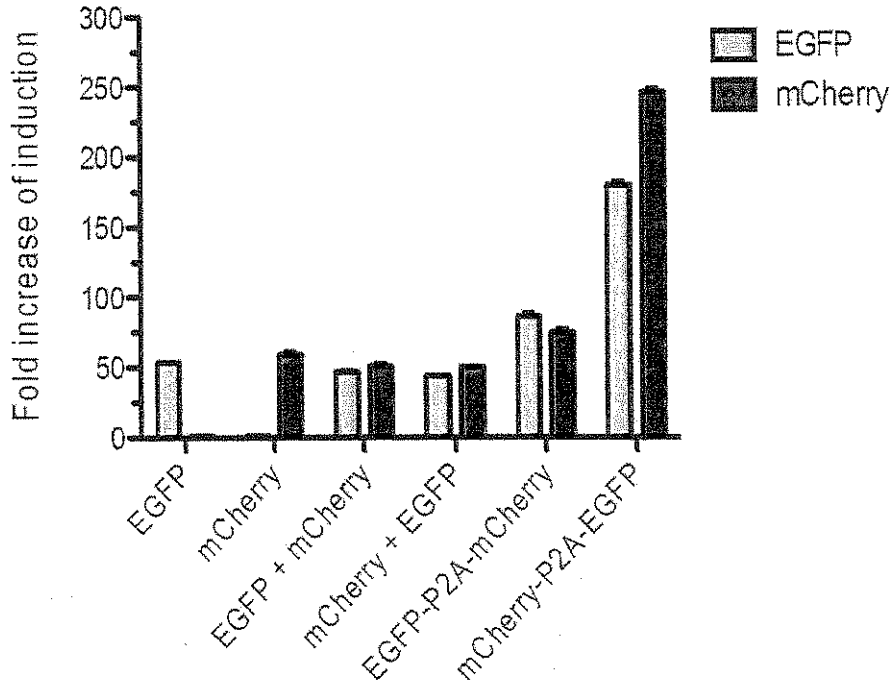


Figure 31

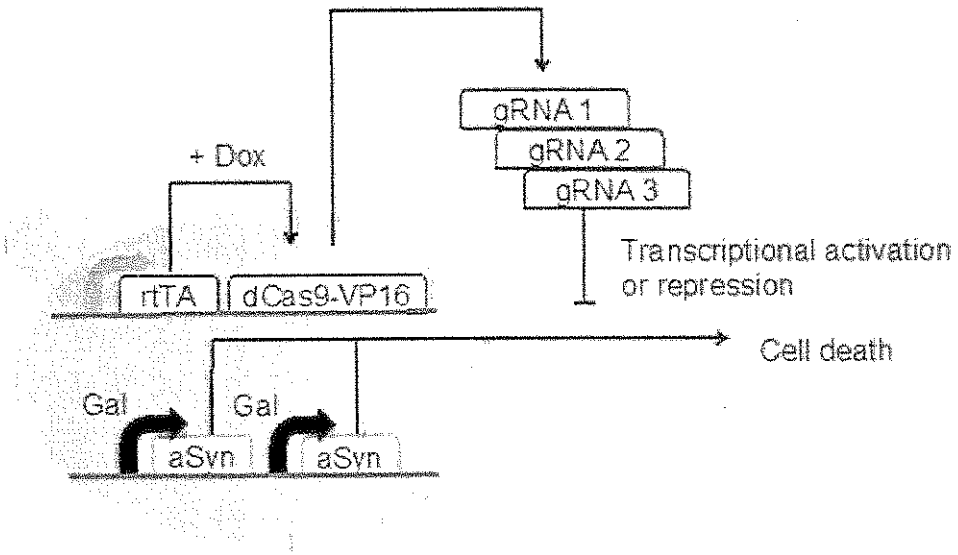


Figure 32

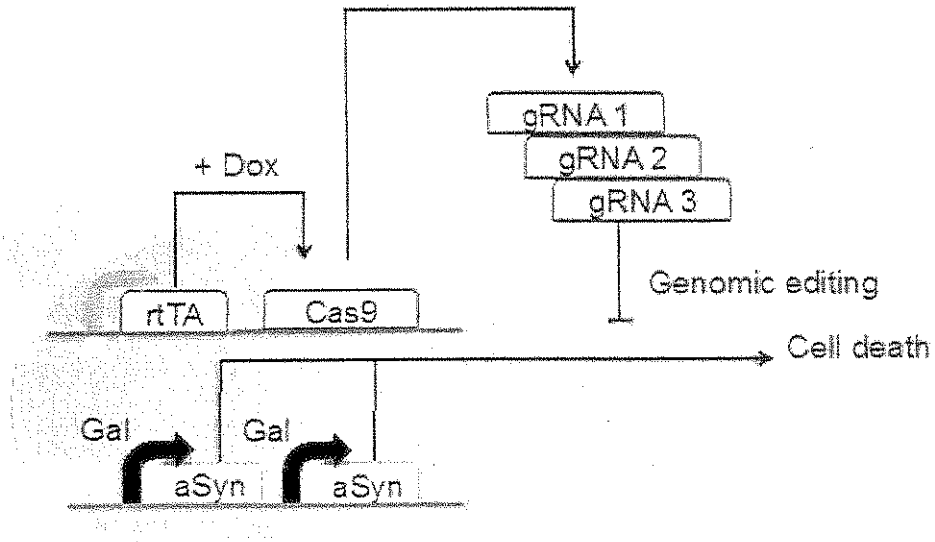


Figure 33

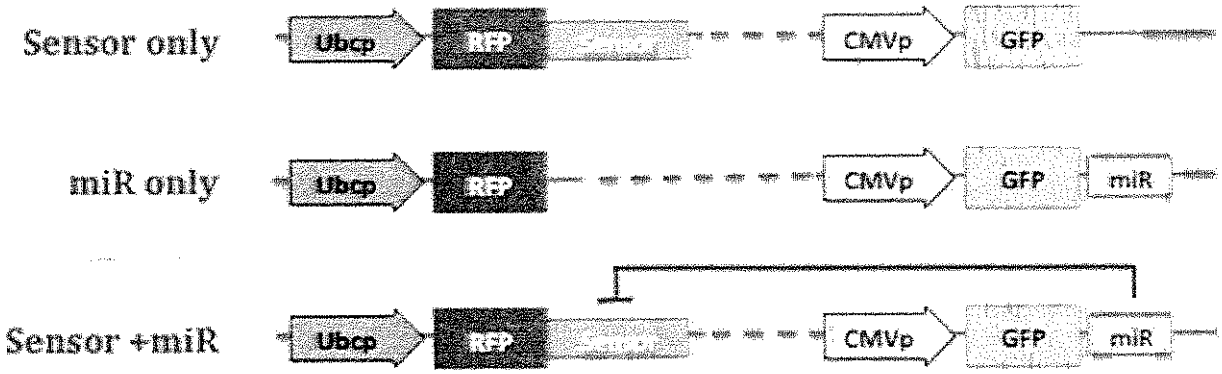

Sensor only
Ubcp
RFP
CMVp
GFP
miR only
Ubcp
RFP
CMVp
GFP
miR
Sensor +miR
Ubcp
RFP
CMVp
GFP
miR

Figure 34

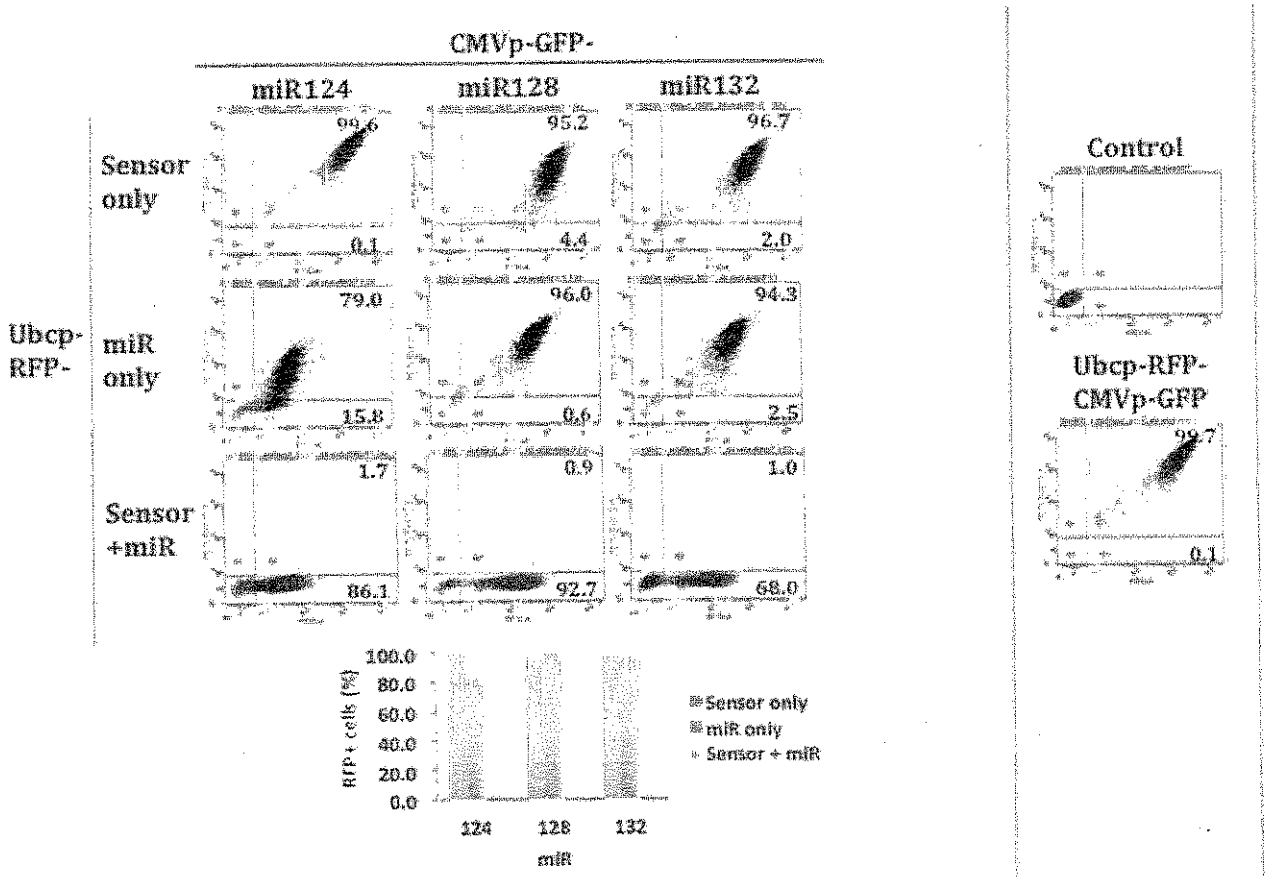

CMVp-GFP-
miR124
miR128
miR132
Sensor only
miR only
Sensor +miR
Ubcp-RFP-
99.6
0.1
95.2
4.4
96.7
2.0
79.0
15.8
96.0
0.6
94.3
2.5
1.7
86.1
0.9
92.7
1.0
68.0
Control
Ubcp-RFP-CMVp-GFP
99.7
0.1
RFP+ cells (%)
100.0
80.0
60.0
40.0
20.0
0.0
124
128
132
miR
Sensor only
miR only
Sensor + miR

Figure 35

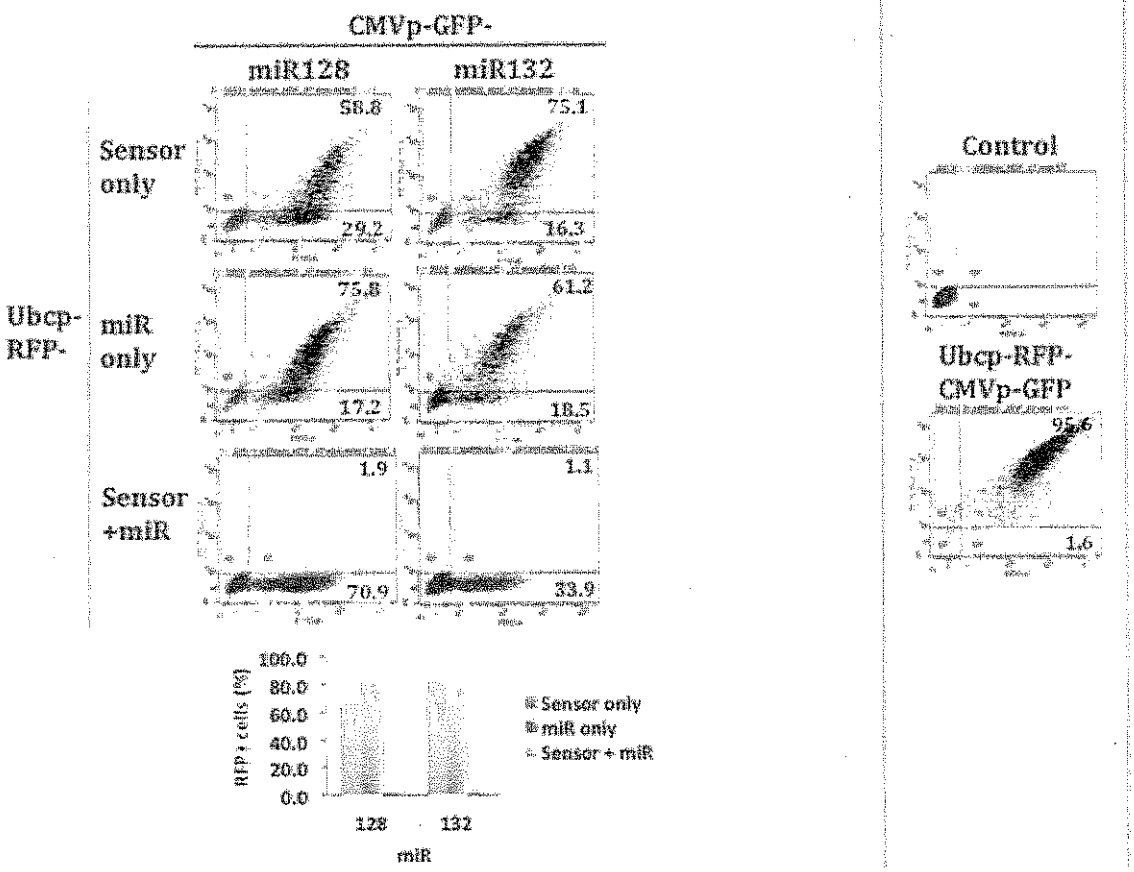

CMVp-GFP-
miR128
miR132
Ubcp-RFP-
Sensor only
miR only
Sensor +miR
58.8
29.2
75.1
16.3
75.8
17.2
61.2
18.5
1.9
70.9
1.1
33.9
Control
Ubcp-RFP-CMVp-GFP
95.6
1.6
RFP+ cells (%)
100.0
80.0
60.0
40.0
20.0
0.0
128
132
miR
Sensor only
miR only
Sensor + miR

Figure 36

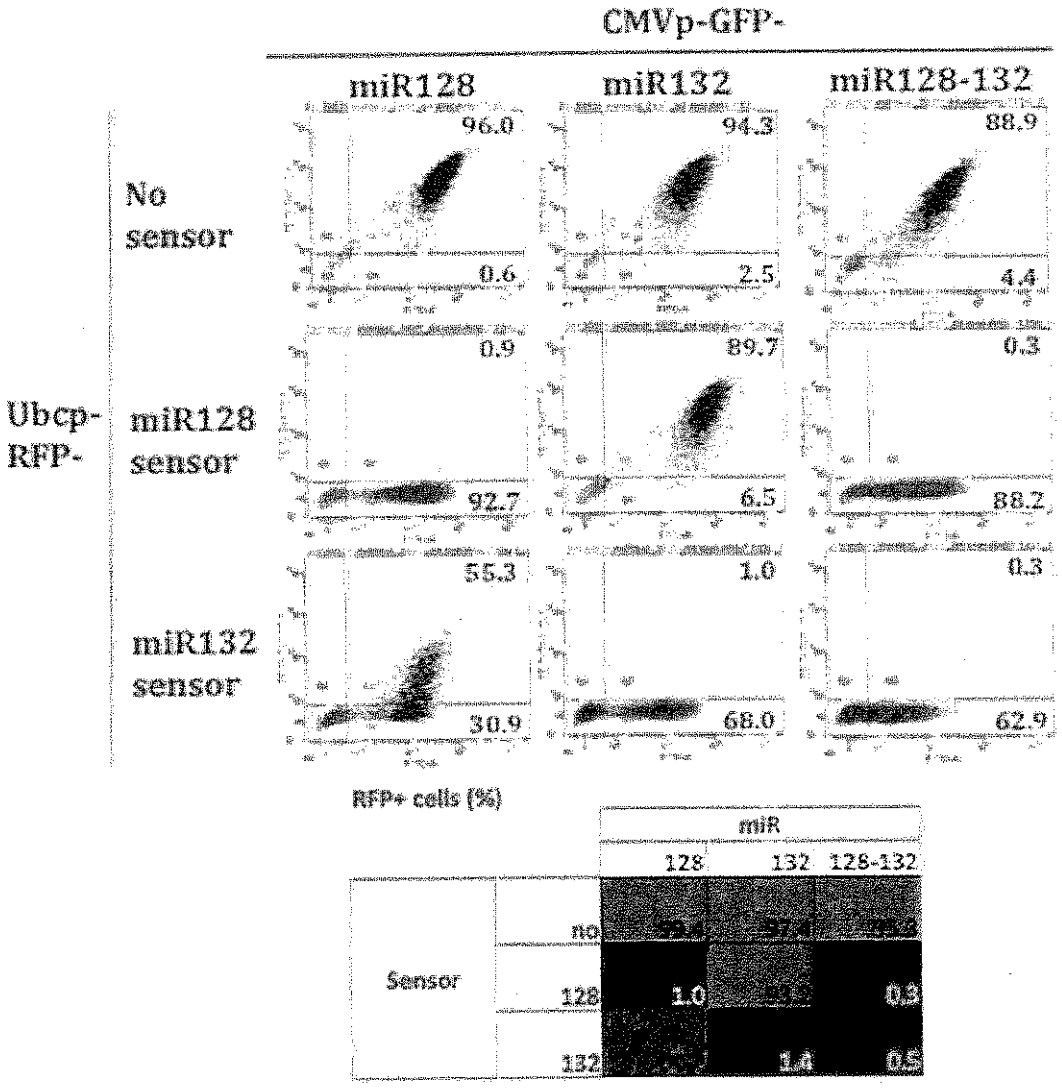

CMVp-GFP-
miR128
miR132
miR128-132
Ubcp-RFP-
No sensor
miR128 sensor
miR132 sensor
96.0
0.6
94.3
2.5
88.9
4.4
0.9
92.7
89.7
6.5
0.3
88.2
55.3
30.9
1.0
68.0
0.3
62.9
RFP+ cells (%)
miR
128
132
128-132
Sensor
no
128
132
1.0
0.3
1.4
0.5

Figure 37

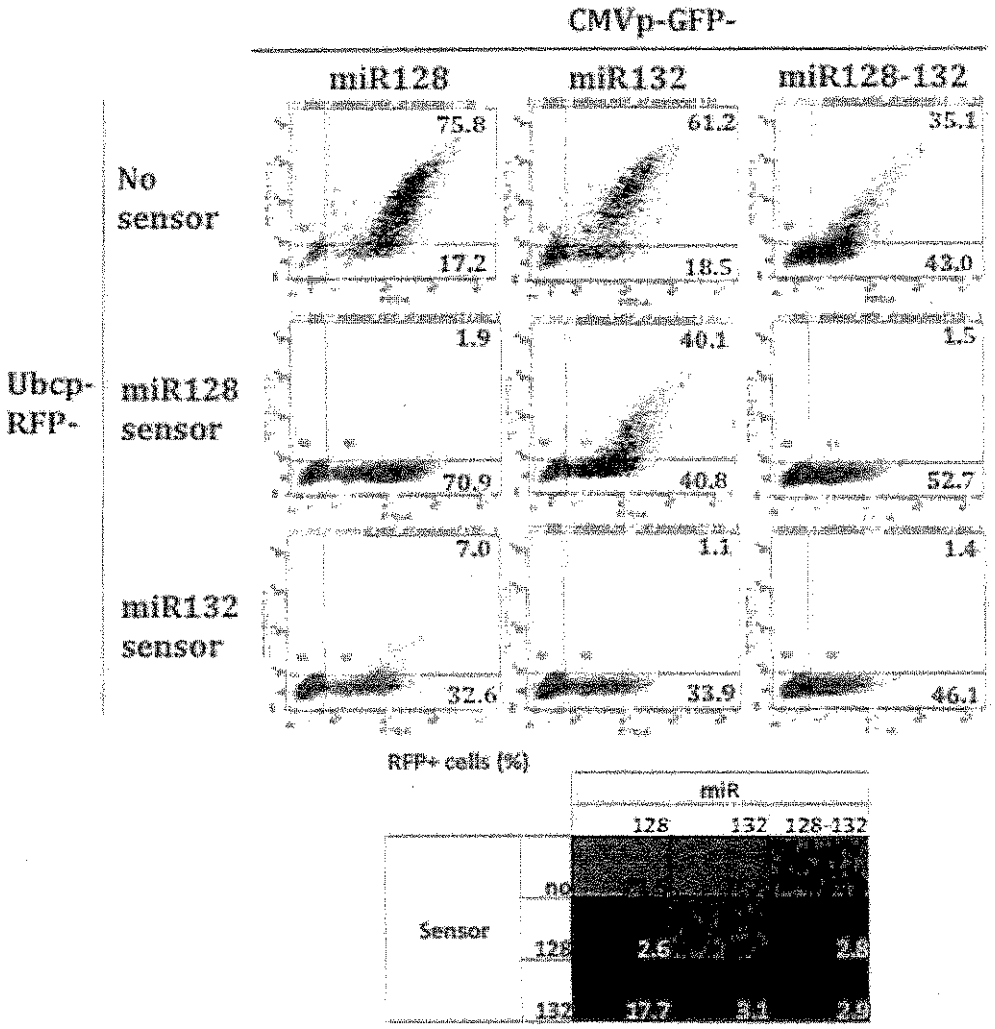
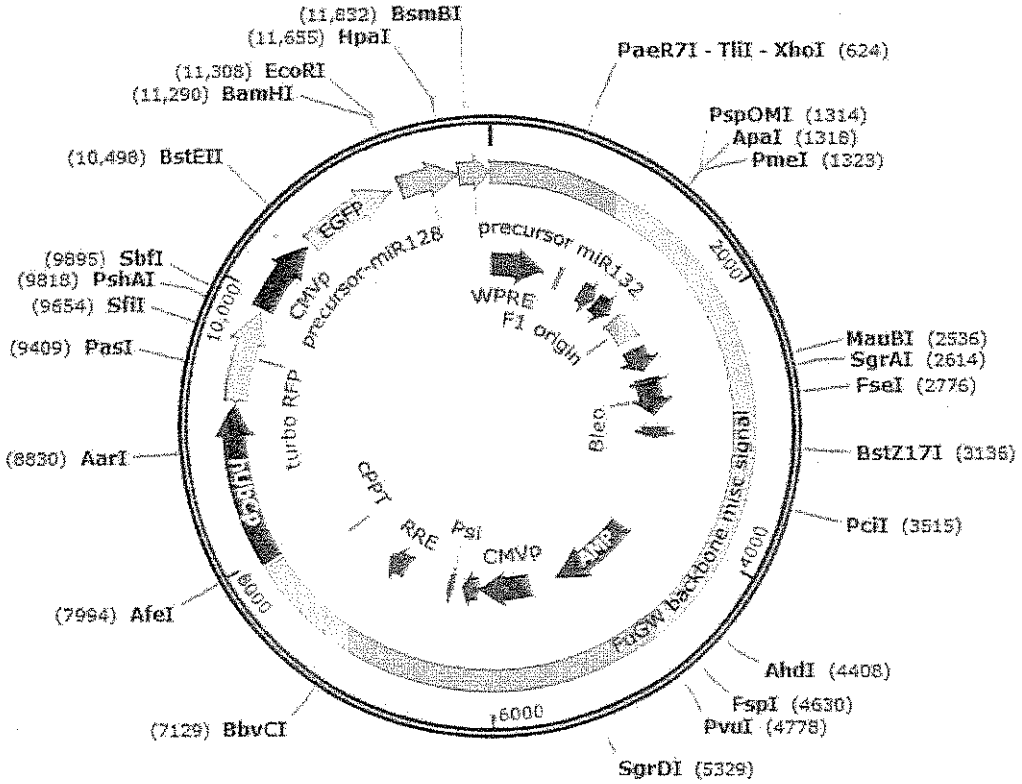

CMVp-GFP-
miR128
miR132
miR128-132
Ubcp-RFP-
No sensor
miR128 sensor
miR132 sensor
75.8
17.2
61.2
18.5
35.1
43.0
1.9
70.9
40.1
40.8
1.5
52.7
7.0
32.6
1.1
33.9
1.4
46.1
RFP+ cells (%)
miR
128
132
128-132
Sensor
no
128
132
2.6
37.7
3.1
2.8
2.0

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
turbo RFP
5'LTR
cPPT
RRE
Psi
CMVp
precursor miR132
WPRE
F1 origin
Bleo
pUC ori
pUC backbone (misc signal)
2000
4000
6000
8000
10,000