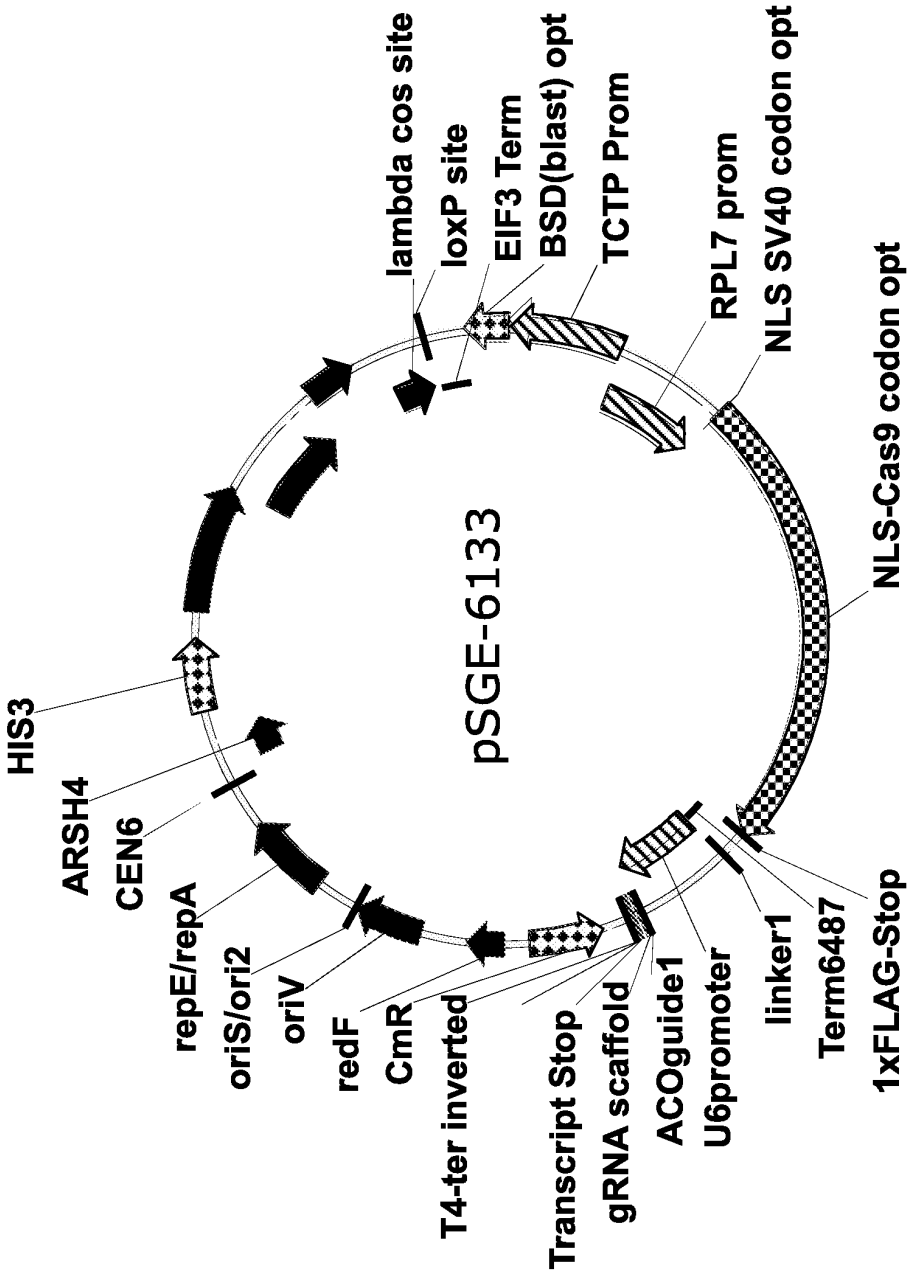

pSGE-6133
lambda cos site
loxP site
EIF3 Term
BSD(blast) opt
TCTP Prom
RPL7 prom
NLS SV40 codon opt
NLS-Cas9 codon opt
1xFLAG-Stop
Term6487
linker1
U6promoter
ACOguide1
gRNA scaffold
Transcript Stop
T4-ter inverted
CmR
redF
oriV
oriS/ori2
repE/repA
CEN6
ARSH4
HIS3

FIG. 1

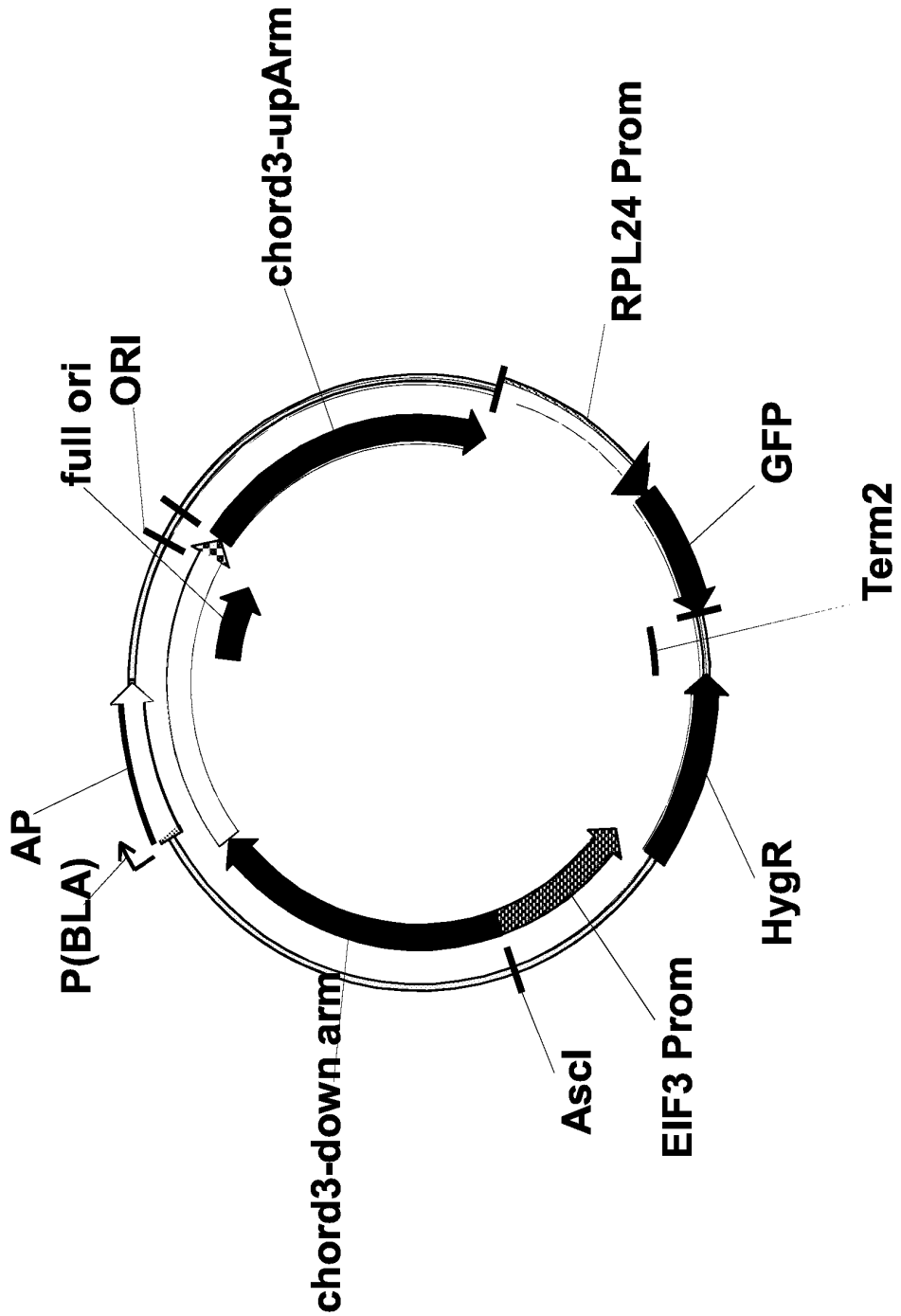

chord3-upArm
RPL24 Prom
GFP
Term2
HygR
EIF3 Prom
AscI
chord3-down arm
P(BLA)
AP
full ori
ORI

FIG. 2

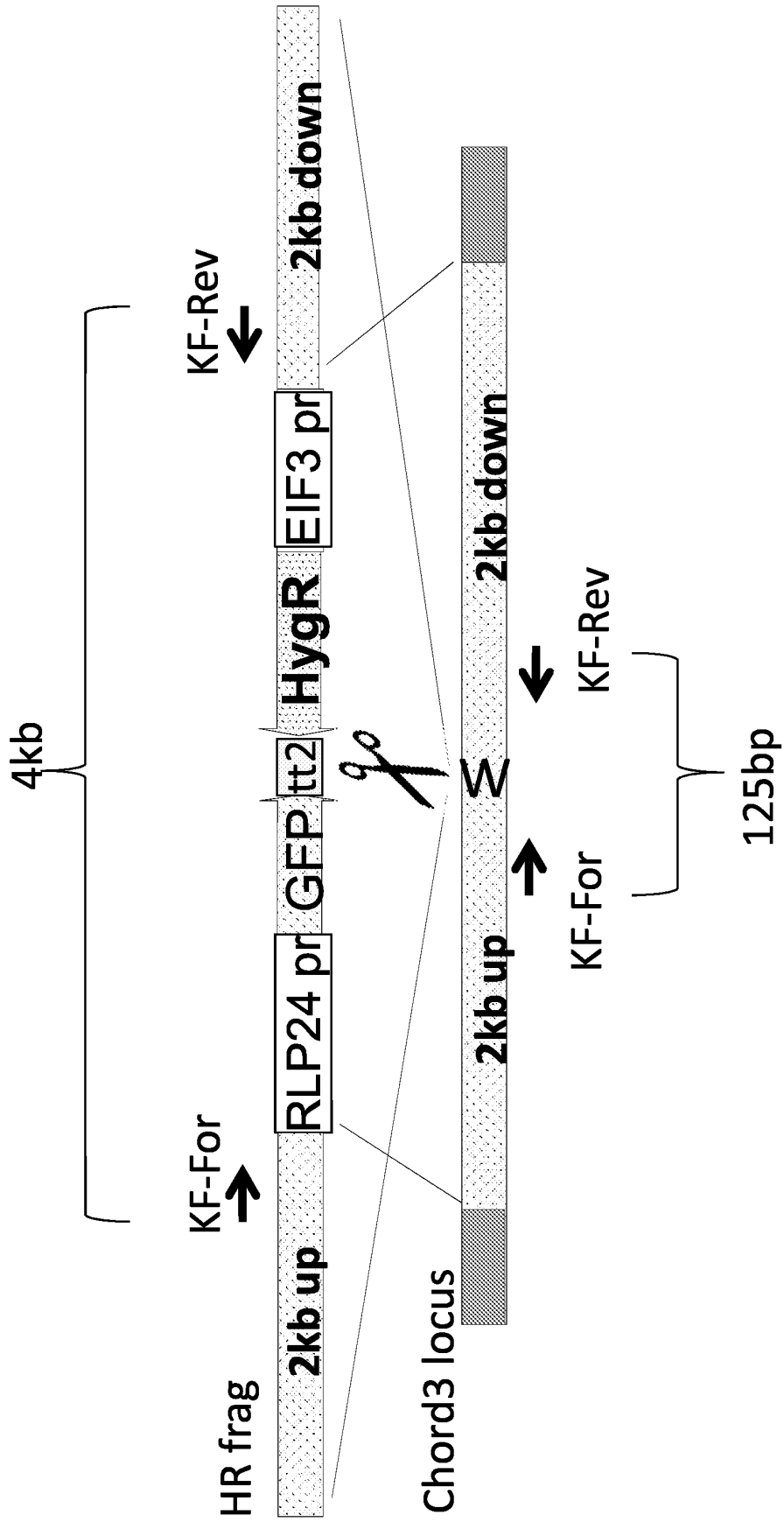

HR frag
2kb up
RLP24 pr
GFP
tt2
HygR
EIF3 pr
2kb down
KF-Rev
KF-For
4kb
Chord3 locus
2kb up
2kb down
KF-For
KF-Rev
125bp

FIG. 3

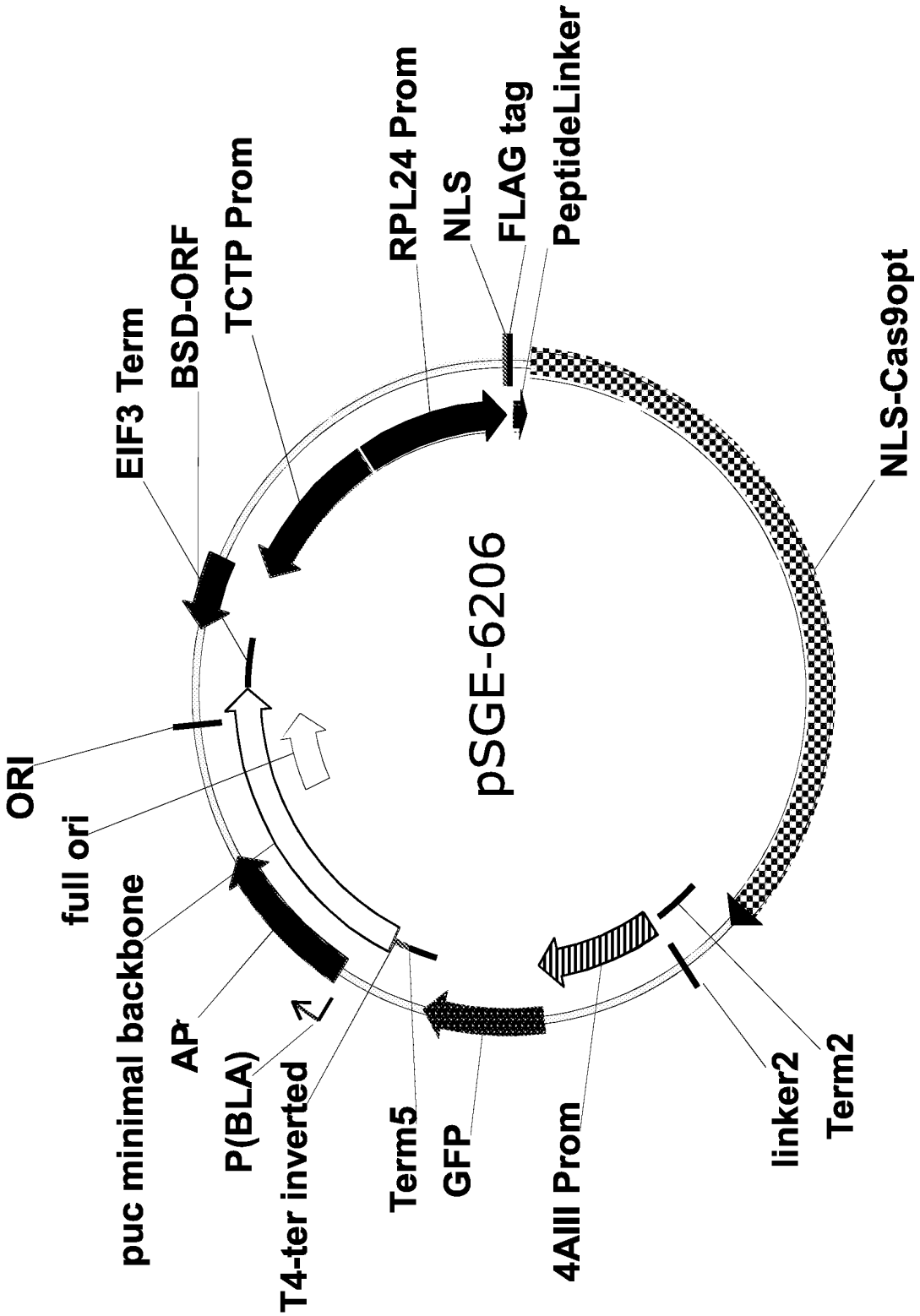
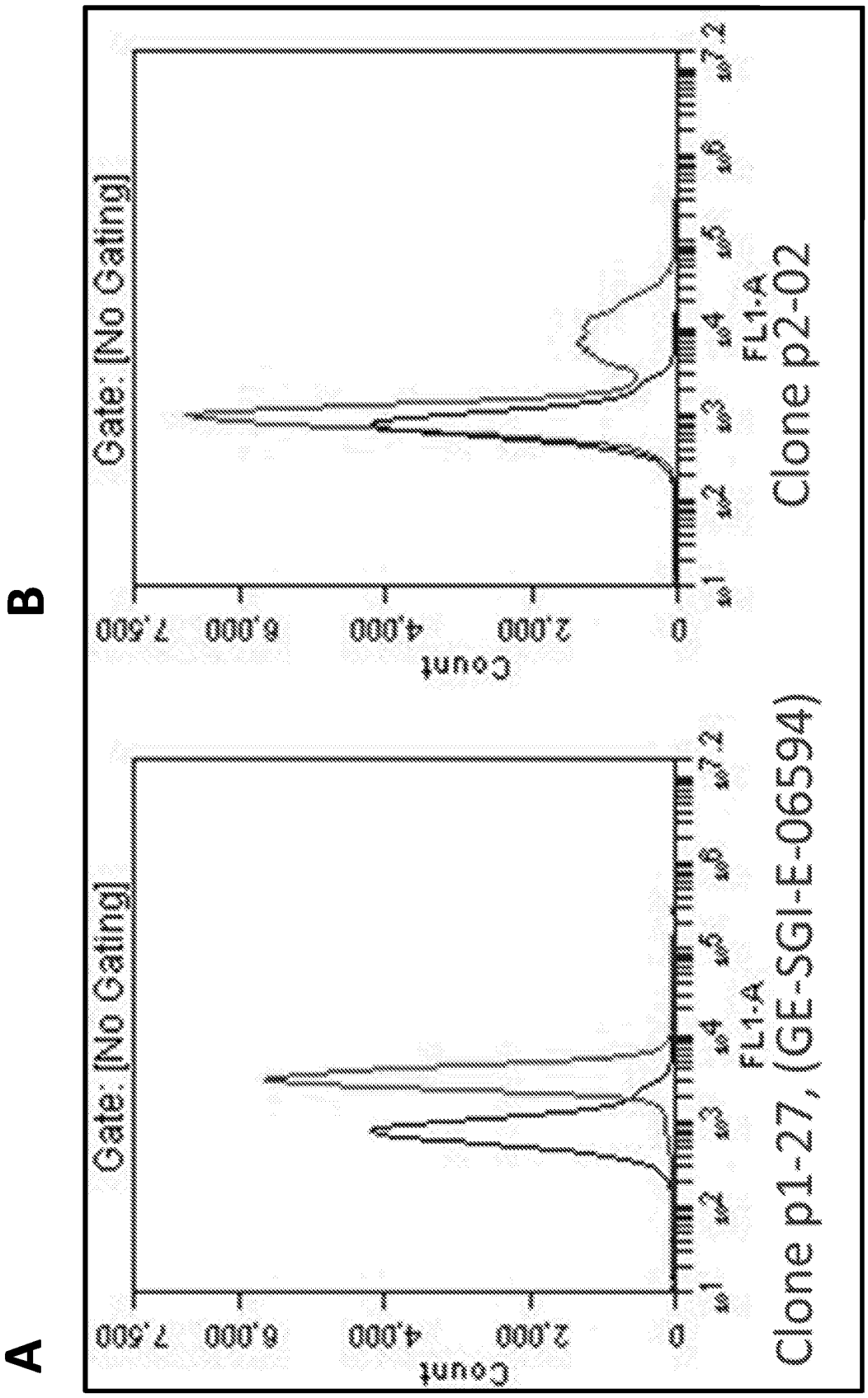

pSGE-6206
ORI
full ori
puc minimal backbone
AP
P(BLA)
T4-ter inverted
Term5
GFP
4AIII Prom
linker2
Term2
NLS-Cas9opt
EIF3 Term
BSD-ORF
TCTP Prom
RPL24 Prom
NLS
FLAG tag
PeptideLinker

FIG. 4


A
Clone p1-27, (GE-SGI-E-06594)
Gate: [No Gating]
Count
0
2,000
4,000
6,000
7,500
FL1-A
B
Clone p2-02
Gate: [No Gating]
Count
0
2,000
4,000
6,000
7,500
FL1-A

FIGS. 5A-5B

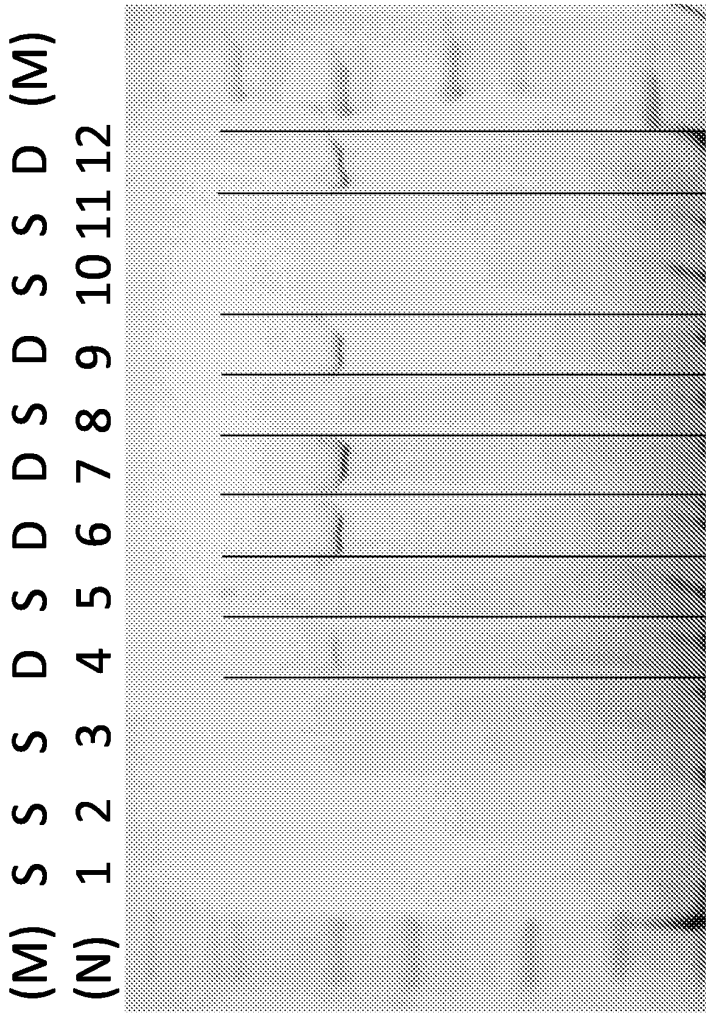


FIG. 6

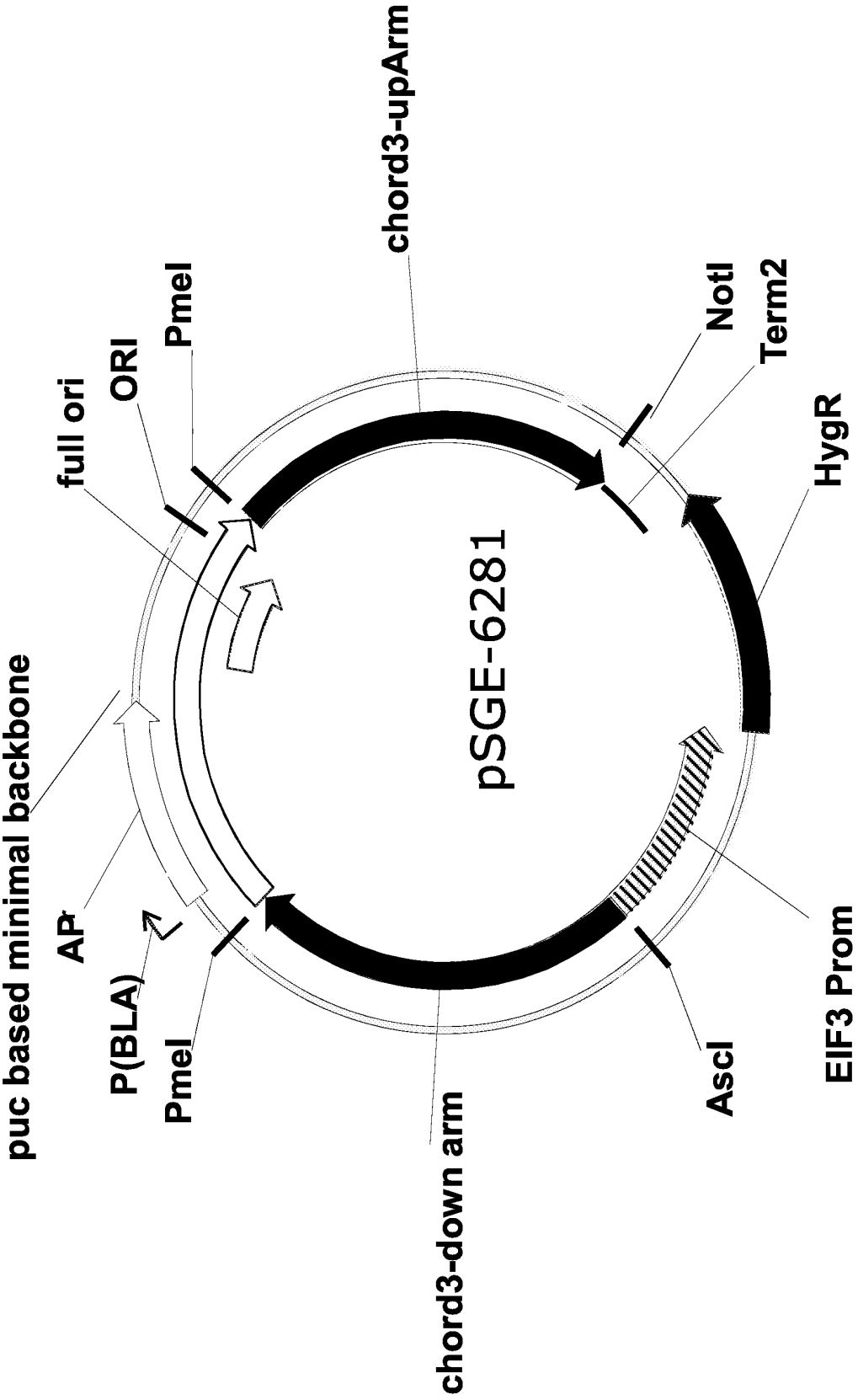

pSGE-6281
chord3-upArm
NotI
Term2
HygR
EIF3 Prom
AscI
chord3-down arm
PmeI
P(BLA)
AP
puc based minimal backbone
full ori
ORI
PmeI

FIG. 7

Nannochloropsis gaditana
+
Trackable Cas9 DNA vector
BlastR cassette
CAS9 cassette
GFP cassette
Blasticidin Selection
Select 100% penetrant lines
Anti-FLAG Western Blot
WE-SGI-E-03730
GE-SGI-E-06791
* GE-SGI-E-06571
GE-SGI-E-06594

FIG. 8

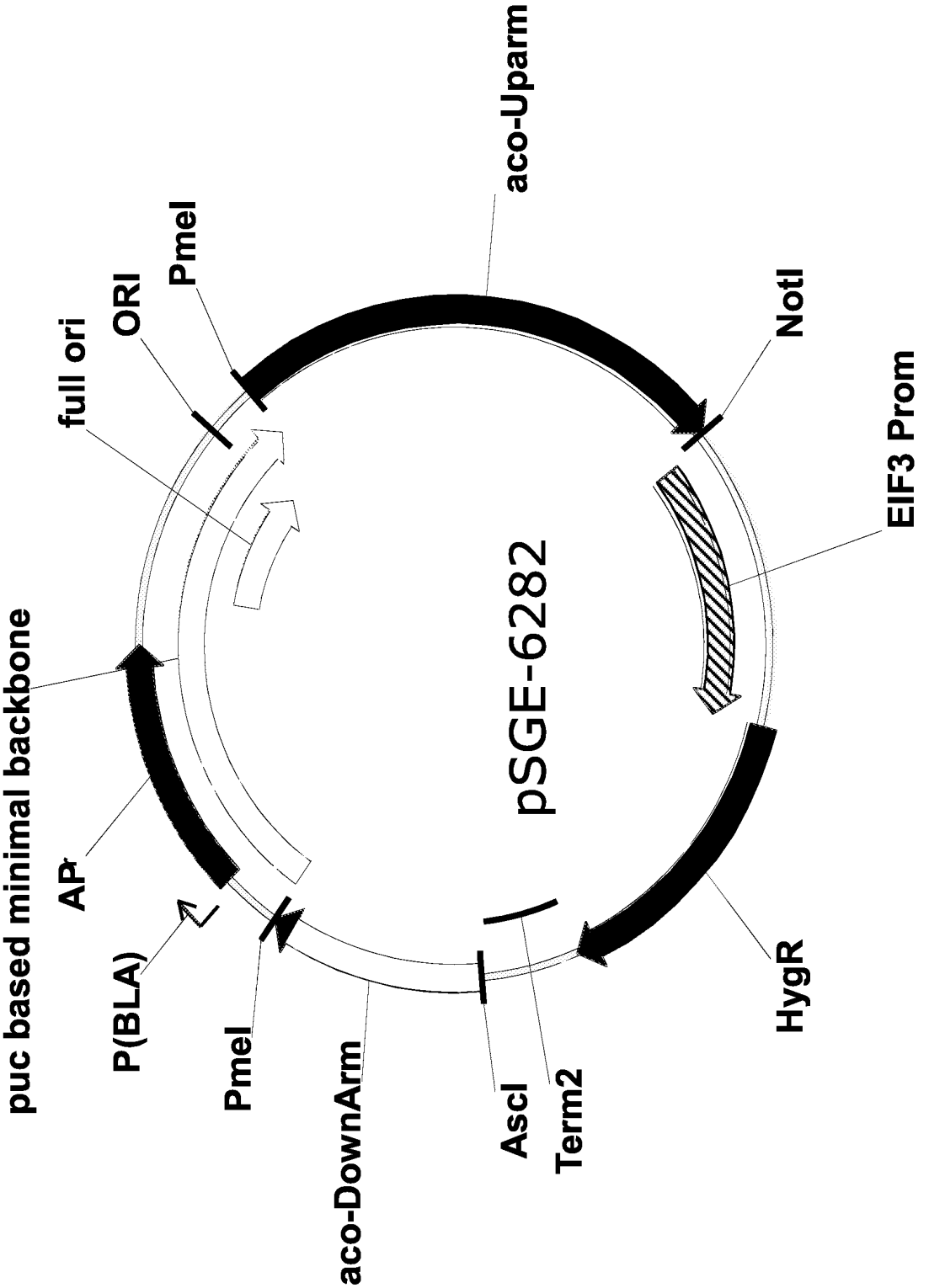

pSGE-6282
aco-Uparm
NotI
EIF3 Prom
HygR
Term2
AscI
aco-DownArm
PmeI
P(BLA)
AP
puc based minimal backbone
full ori
ORI
PmeI

FIG. 9

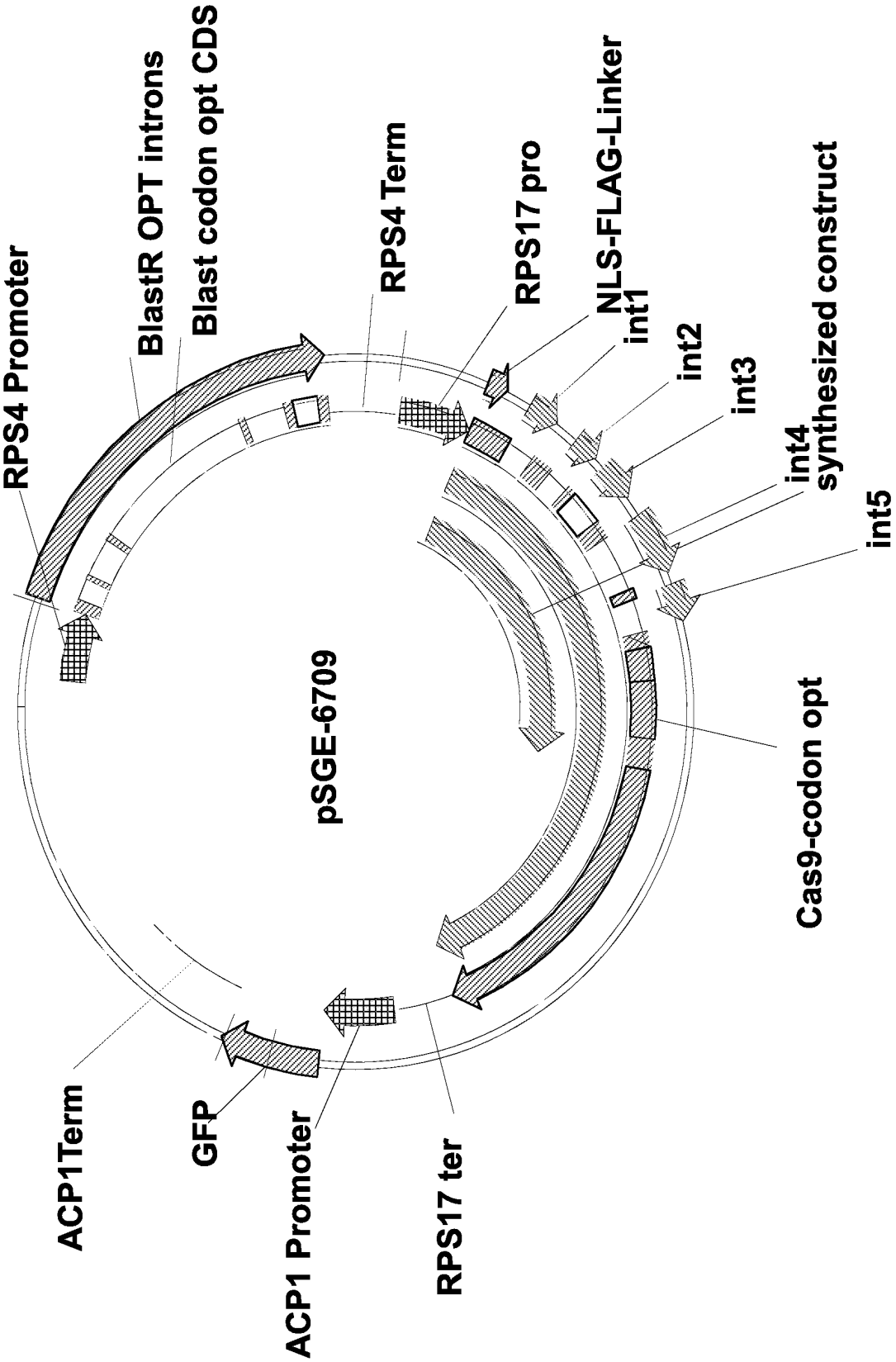

RPS4 Promoter
BlastR OPT introns
Blast codon opt CDS
RPS4 Term
RPS17 pro
NLS-FLAG-Linker
int1
int2
int3
int4
synthesized construct
int5
Cas9-codon opt
pSGE-6709
ACP1Term
GFP
ACP1 Promoter
RPS17 ter

FIG. 10

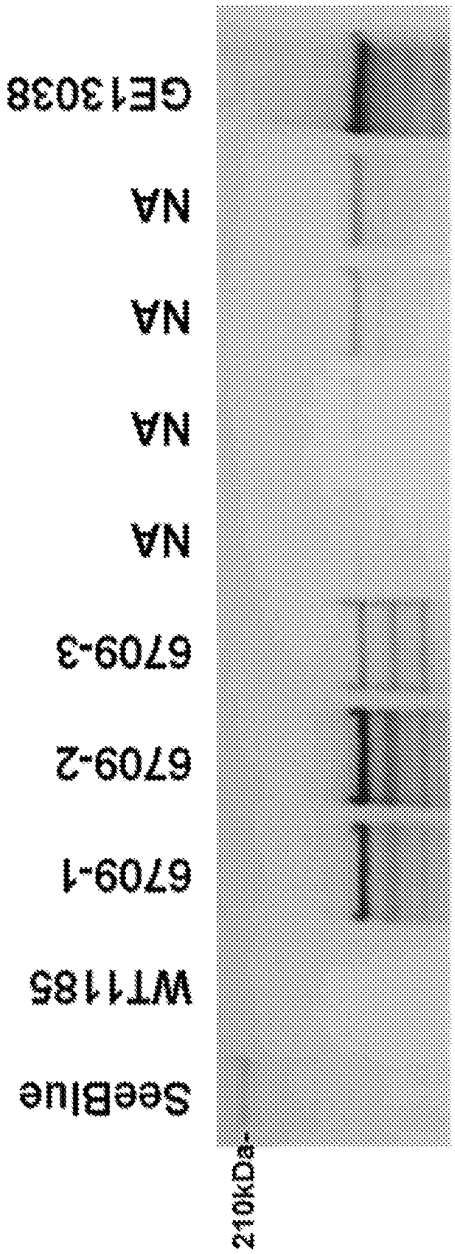

210kDa-
α-Cas9
SeeBlue
WT1185
6709-1
6709-2
6709-3
NA
NA
NA
NA
GE13038

FIG. 11

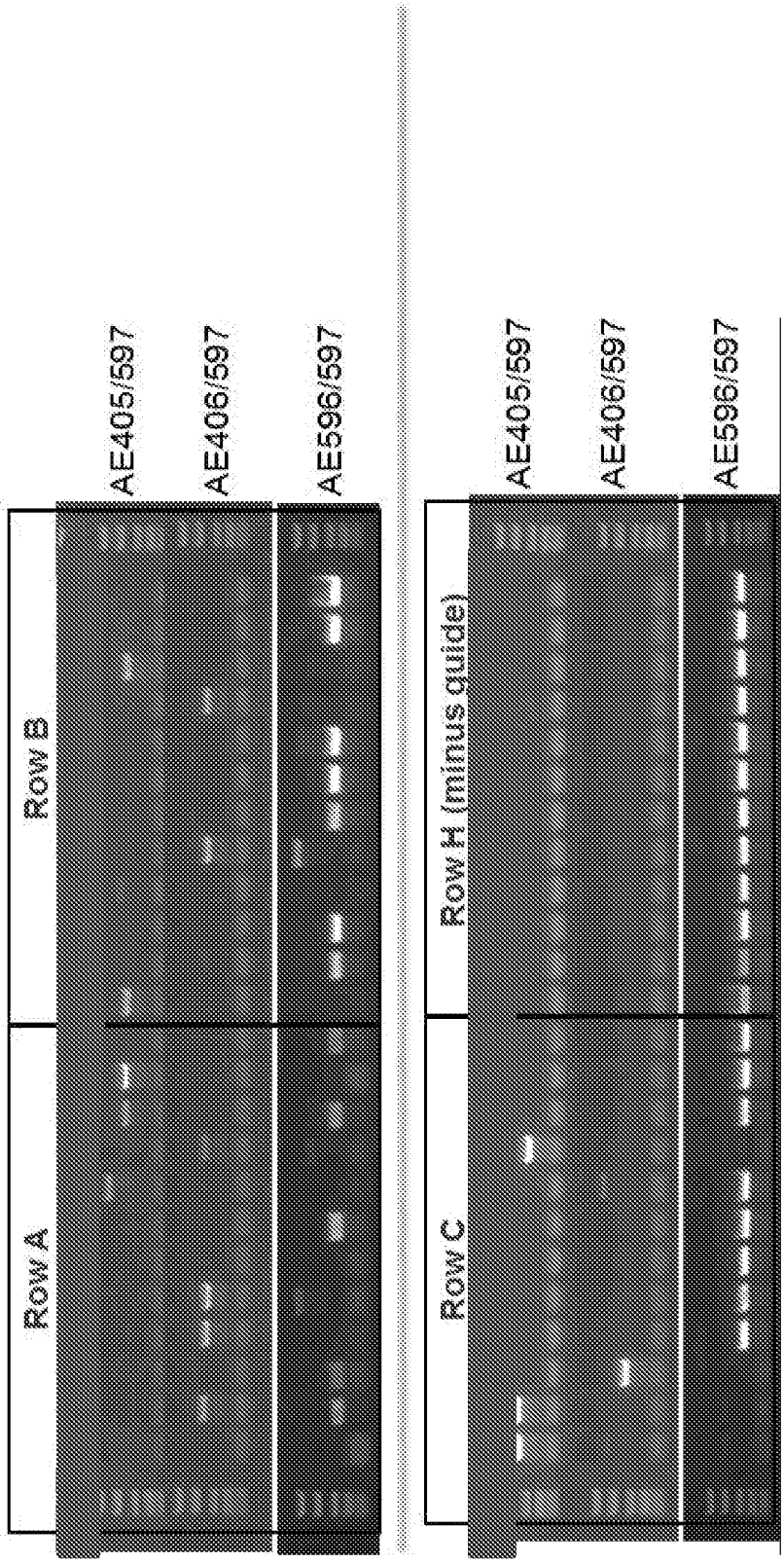
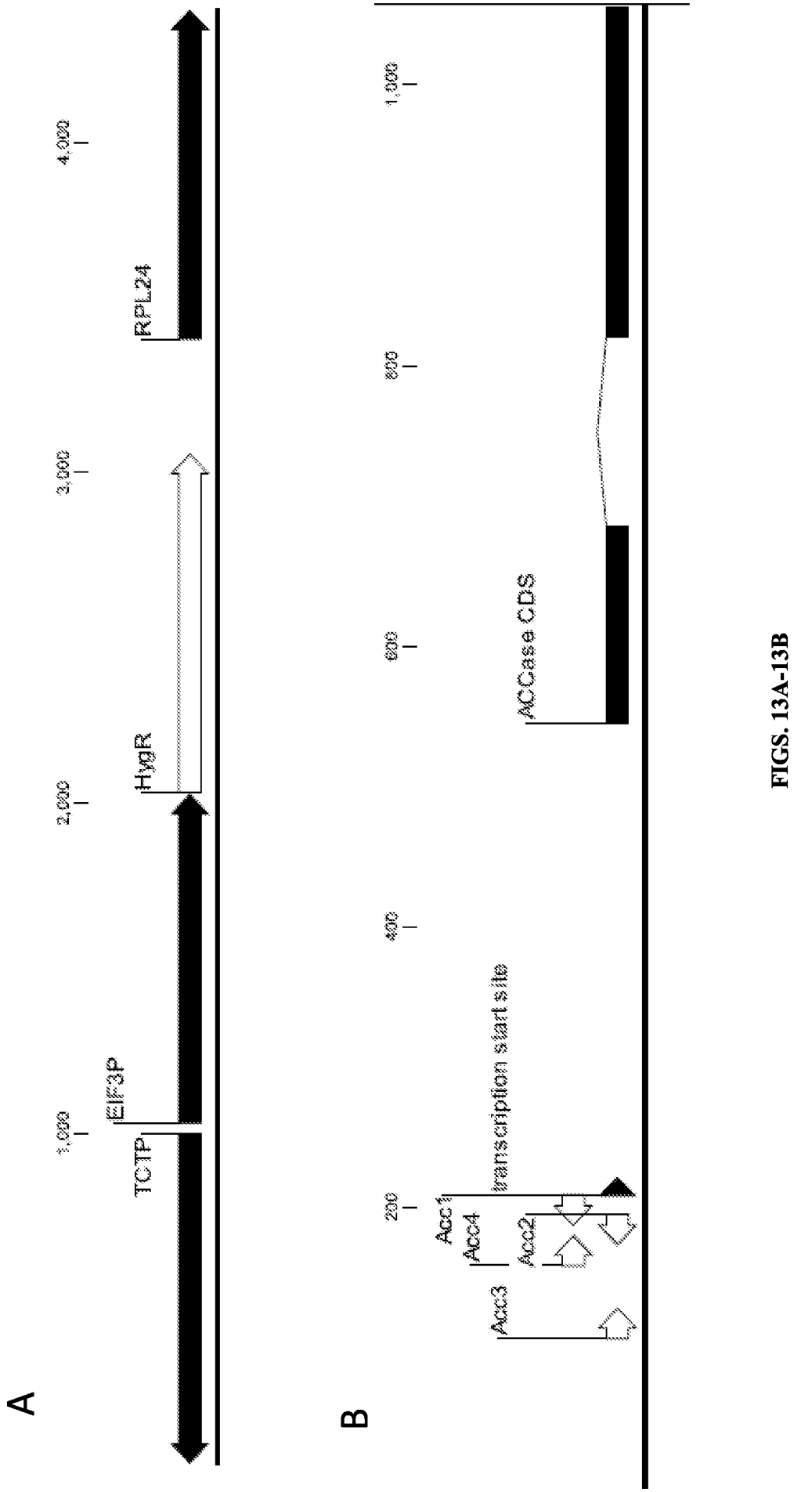
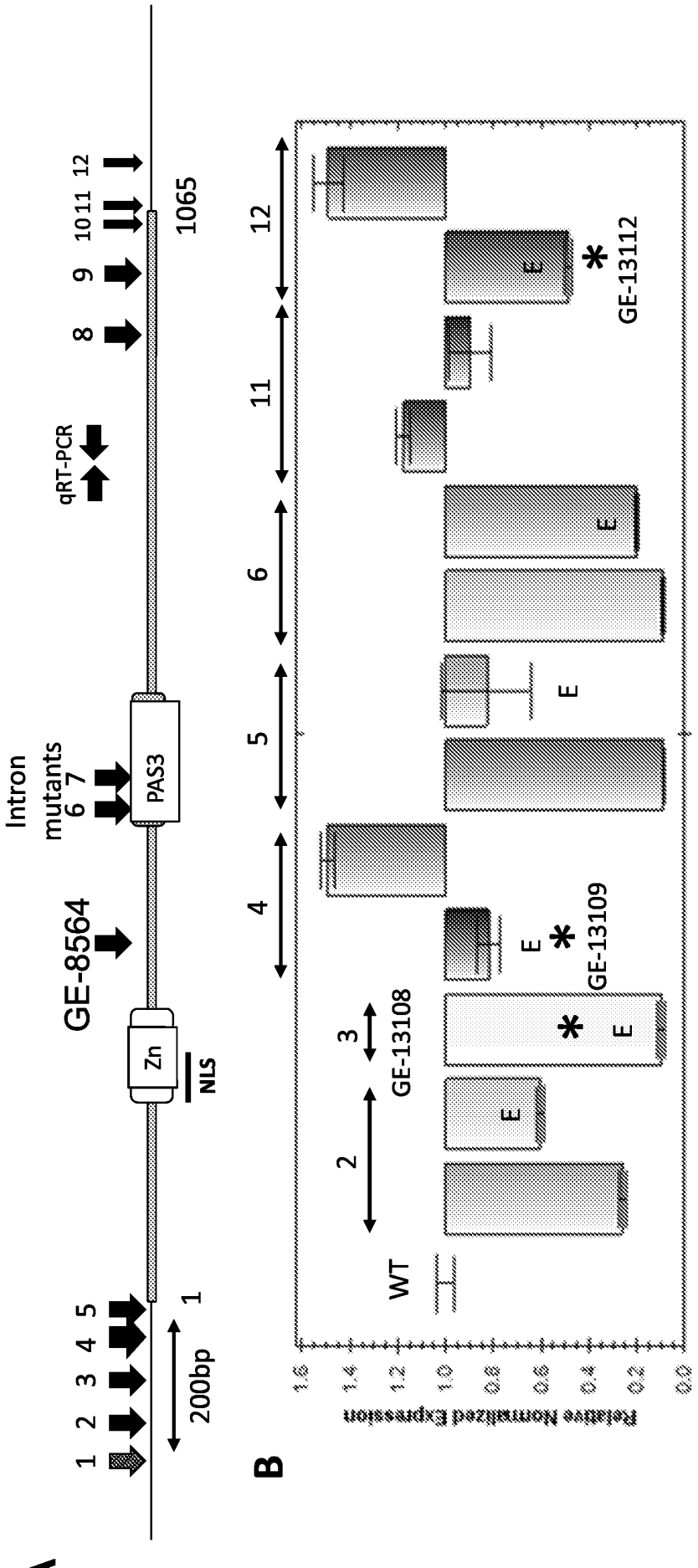

Row A
Row B
Row C
Row H (minus guide)
AE405/597
AE406/597
AE596/597
AE405/597
AE406/597
AE596/597

FIG. 12


A
1,000
2,000
3,000
4,000
TCTP
EIF3P
HygR
RPL24
B
200
400
600
800
1,000
Acc1
Acc4
Acc2
Acc3
transcription start site
ACCase CDS

FIGS. 13A-13B


A
1 2 3 4 5
200bp
1
1065
8 9 10 11 12
qRT-PCR
Intron mutants 6 7
PAS3
GE-8564
Zn
NLS
B
Relative Normalized Expression
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WT
2
3
GE-13108
4
E
GE-13109
5
E
6
E
11
E
12
GE-13112

FIGS. 14A-14B

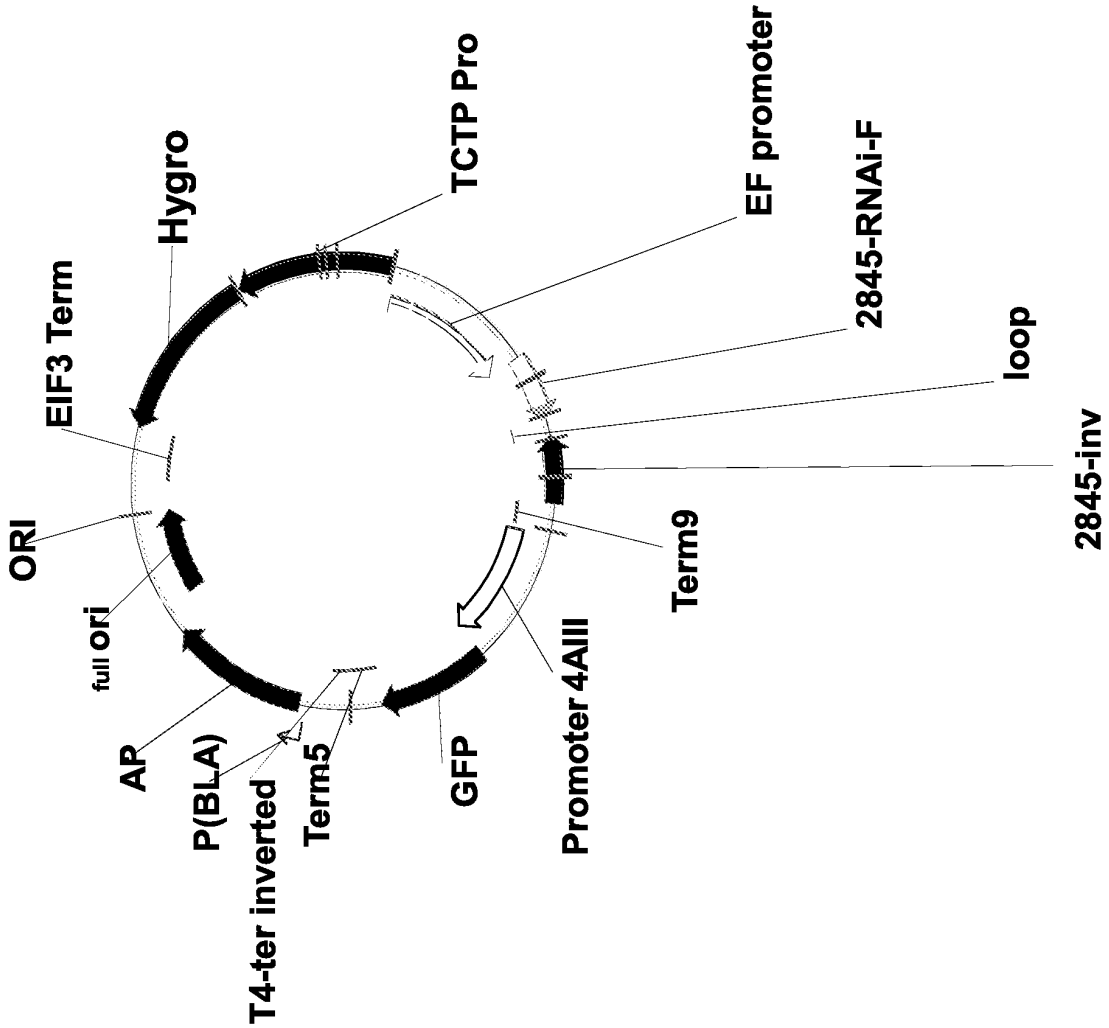

TCTP Pro
EF promoter
2845-RNAi-F
loop
2845-inv
Term9
Promoter 4AIII
GFP
Term5
T4-ter inverted
P(BLA)
AP
full ori
ORI
EIF3 Term
Hygro

FIG. 15

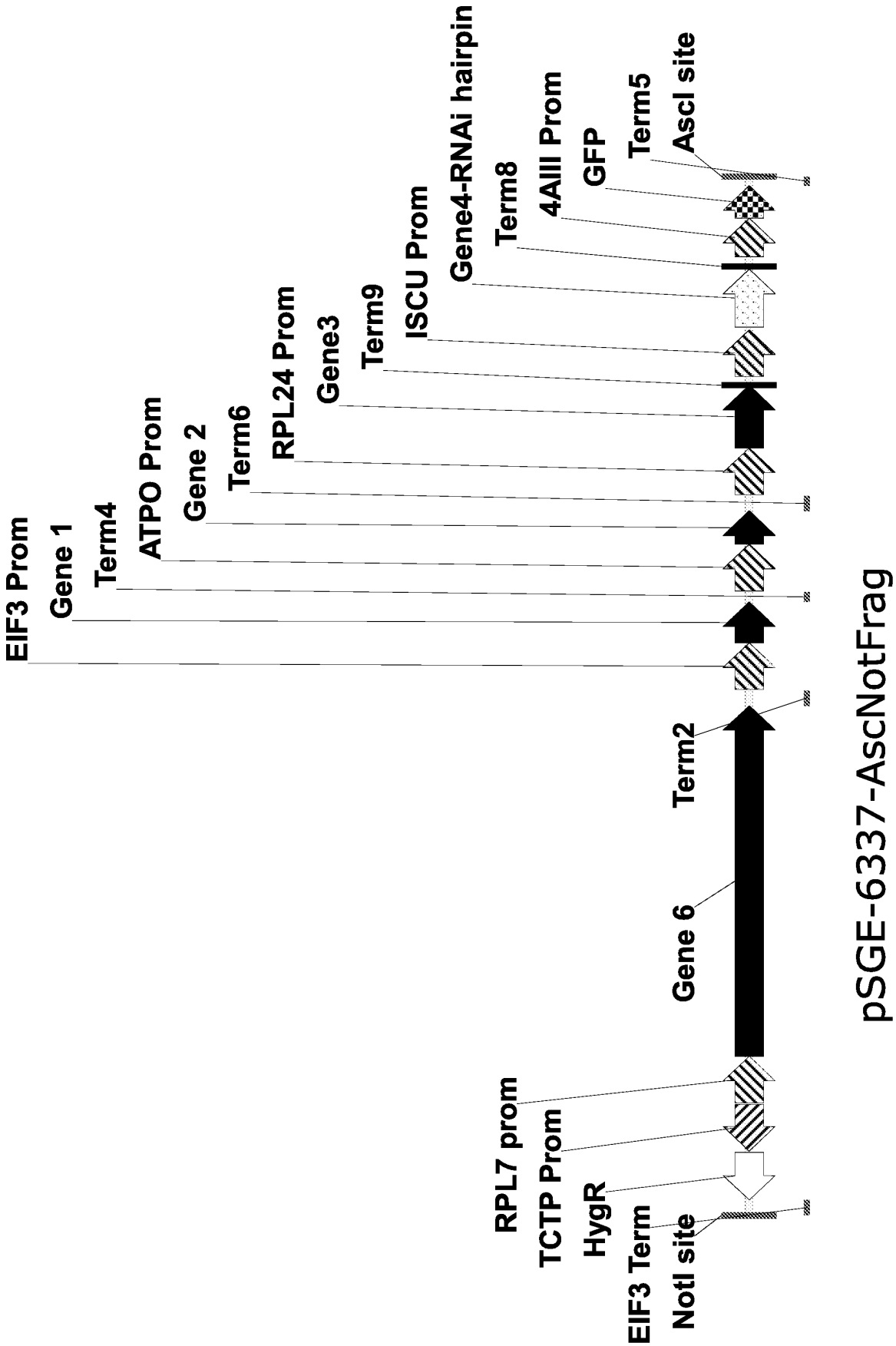

EIF3 Prom
Gene 1
Term4
ATPO Prom
Gene 2
Term6
RPL24 Prom
Gene3
Term9
ISCU Prom
Gene4-RNAi hairpin
Term8
4AIII Prom
GFP
Term5
AscI site
Term2
Gene 6
RPL7 prom
TCTP Prom
HygR
EIF3 Term
NotI site
pSGE-6337-AscNotFrag

FIG. 16

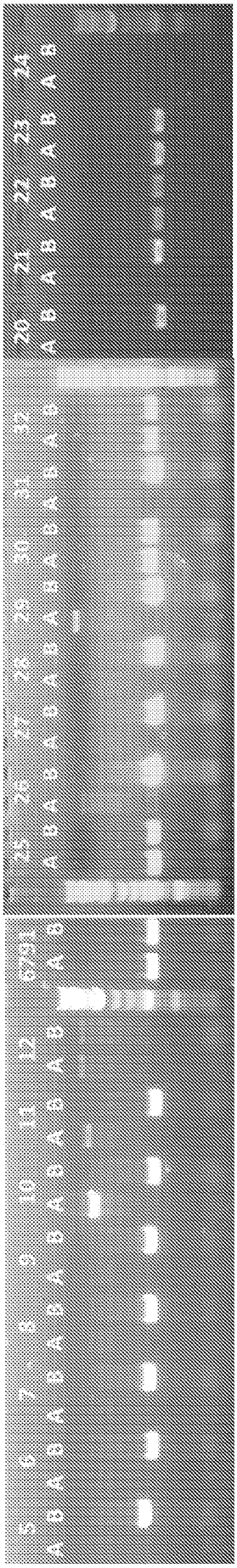


FIG. 17

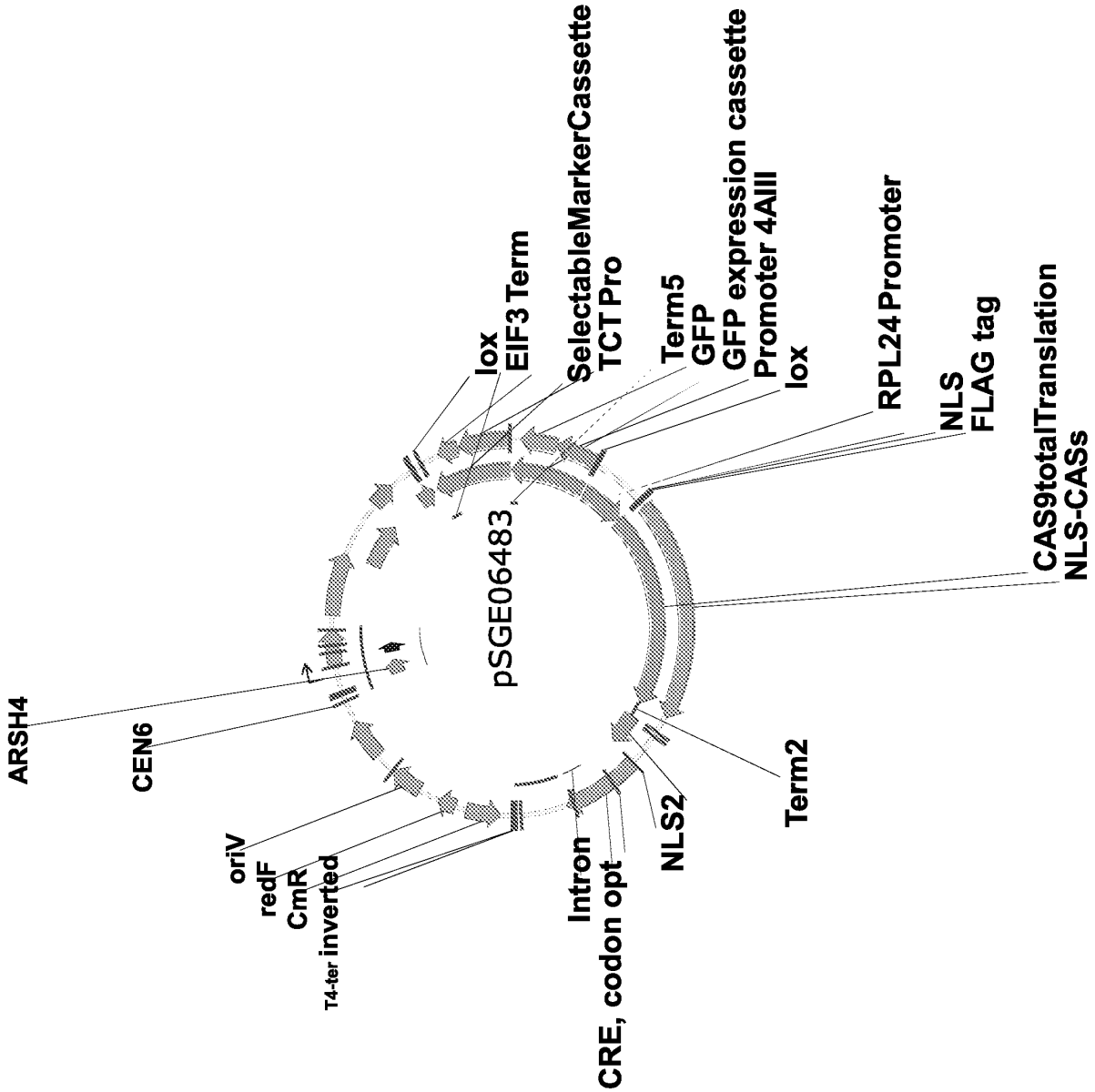

pSGE06483
ARSH4
CEN6
oriV
redF
CmR
T4-ter inverted
Intron
CRE, codon opt
NLS2
Term2
CAS9totalTranslation
NLS-CASs
NLS
FLAG tag
RPL24 Promoter
lox
GFP expression cassette
Promoter 4AIII
Term5
GFP
SelectableMarkerCassette
TCT Pro
lox
EIF3 Term

FIG. 18

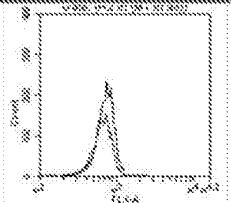
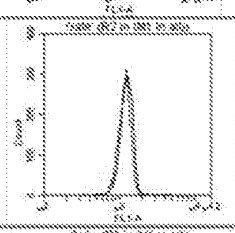
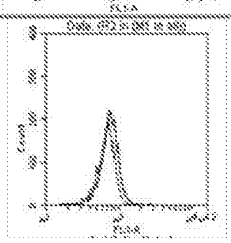
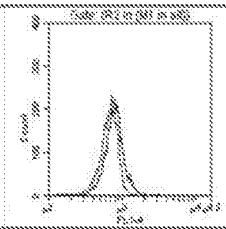
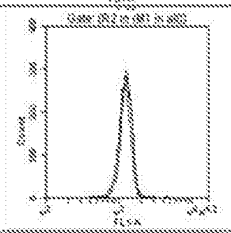
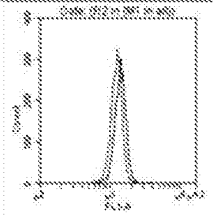
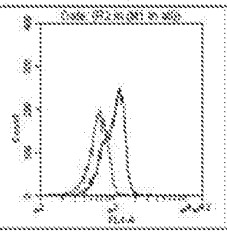
Sample		Mean Fluorescence (GFP)	
		NH_4	NO_2
WE-SGI-E-03730		728	661
pSGE06483-A11		1262	1180
pSGE06483-B11		773	683
pSGE06483-C12		807	667
pSGE06483-D12		1266	1195
pSGE06483-E12		1415	1248
pSGE06483-F12		1042	637

FIG. 19

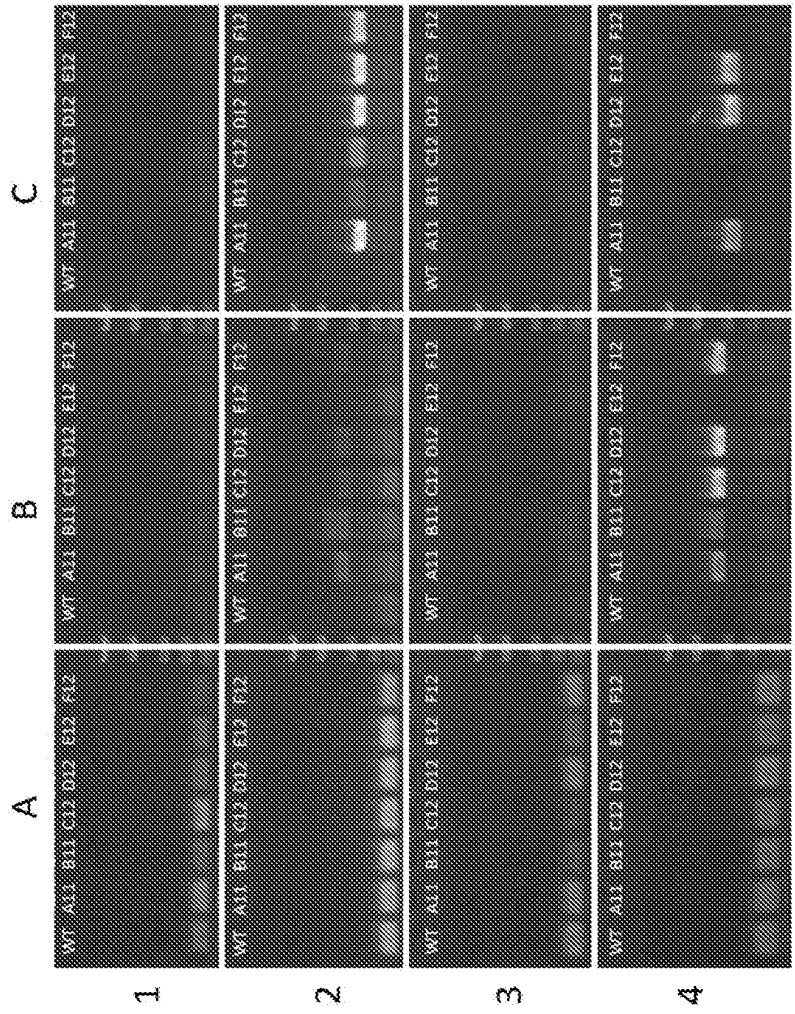


FIG. 20

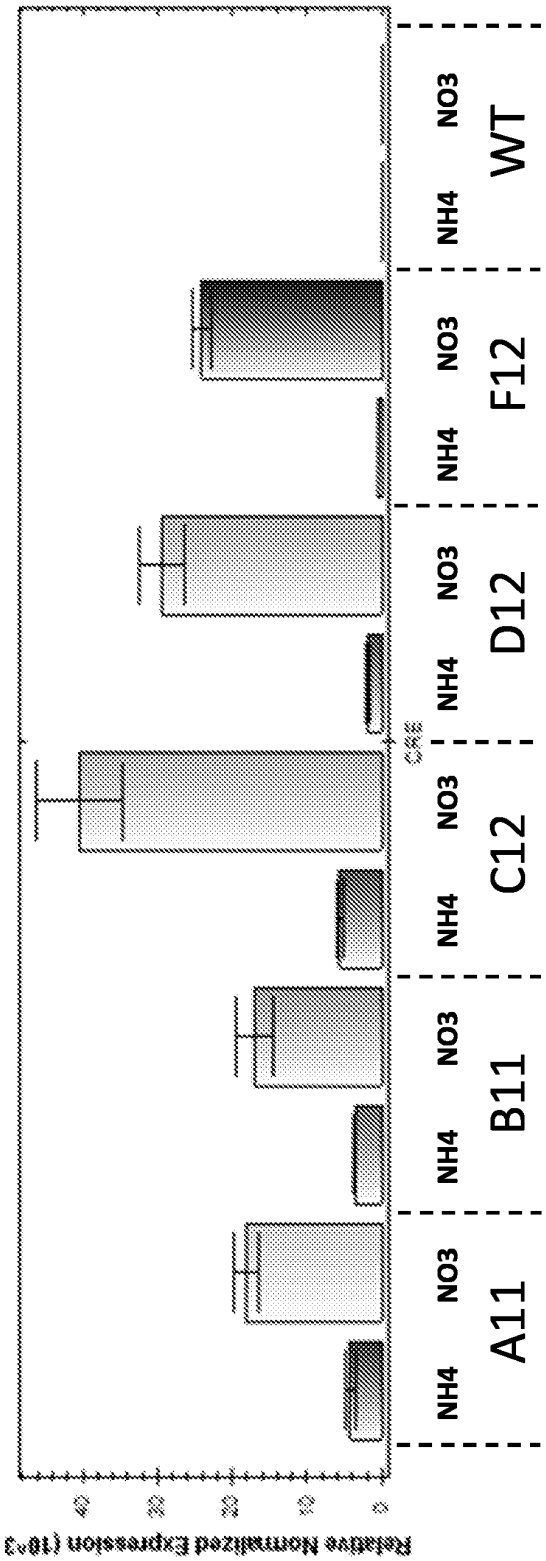

Relative Normalized Expression (10^3)
0
20
40
60
A11
B11
C12
D12
F12
WT
NH4
NO3
SE

FIG. 21

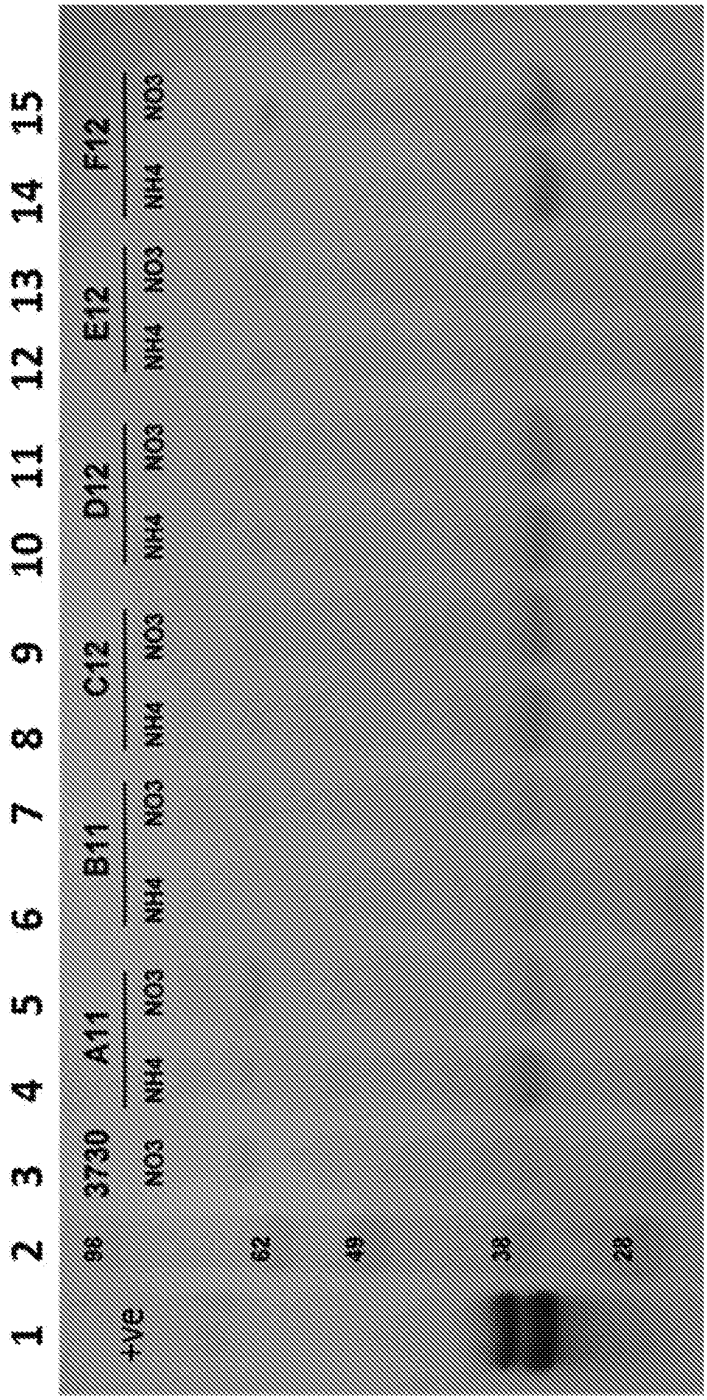

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
98
3730
+Ve
NO3
A11
NH4
NO3
B11
NH4
NO3
C12
NH4
NO3
D12
NH4
NO3
E12
NH4
NO3
F12
NH4
NO3
62
49
38
28

FIG. 22

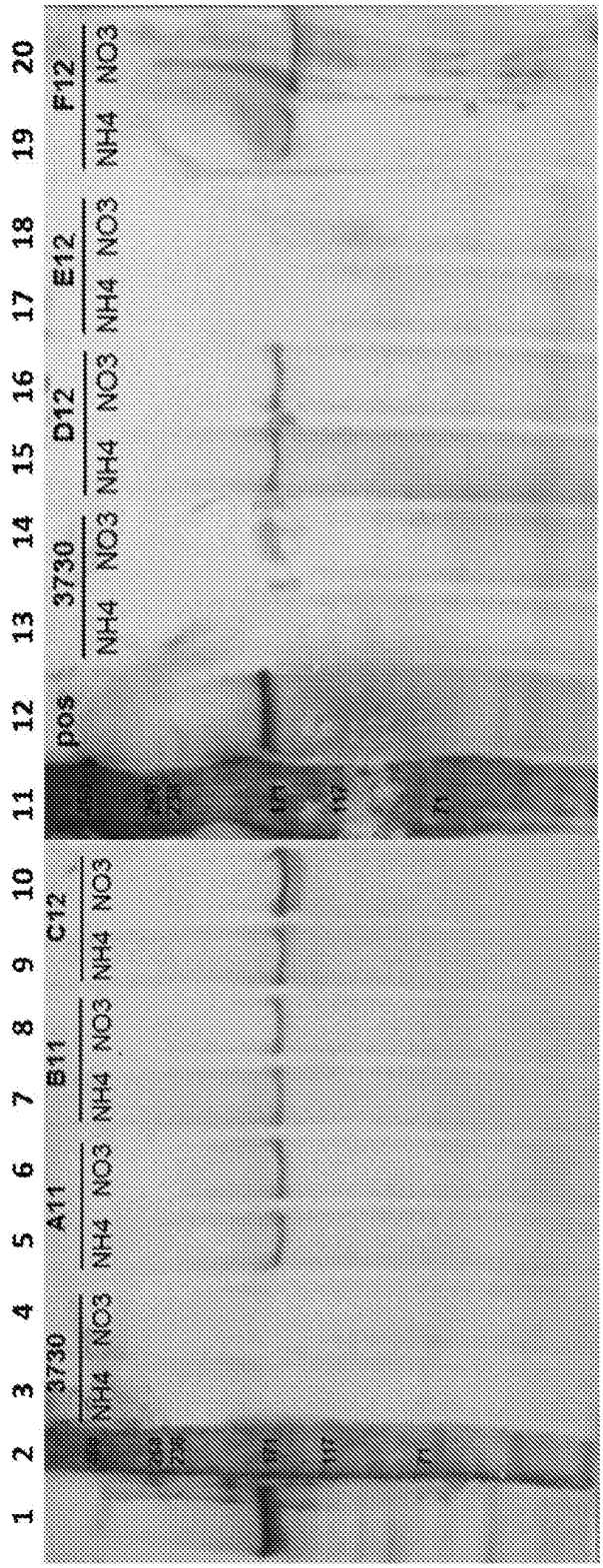

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
3730
NH4
NO3
A11
NH4
NO3
B11
NH4
NO3
C12
NH4
NO3
pos
3730
NH4
NO3
D12
NH4
NO3
E12
NH4
NO3
F12
NH4
NO3

FIG. 23

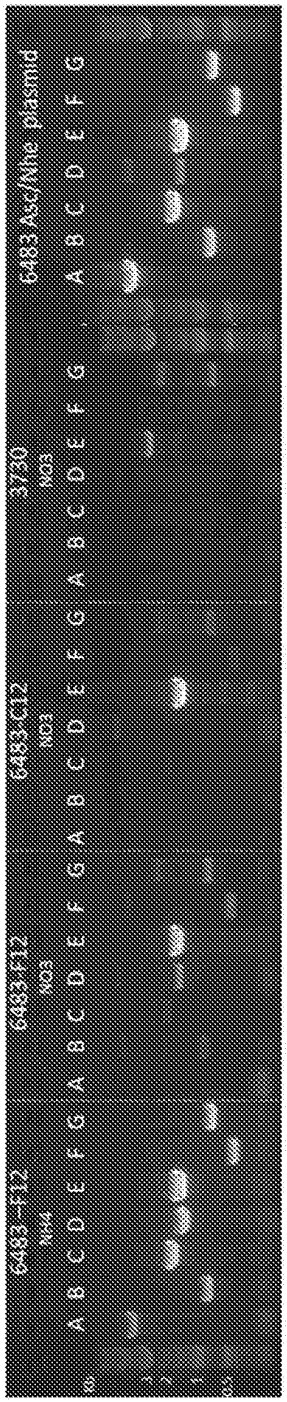


FIG. 24

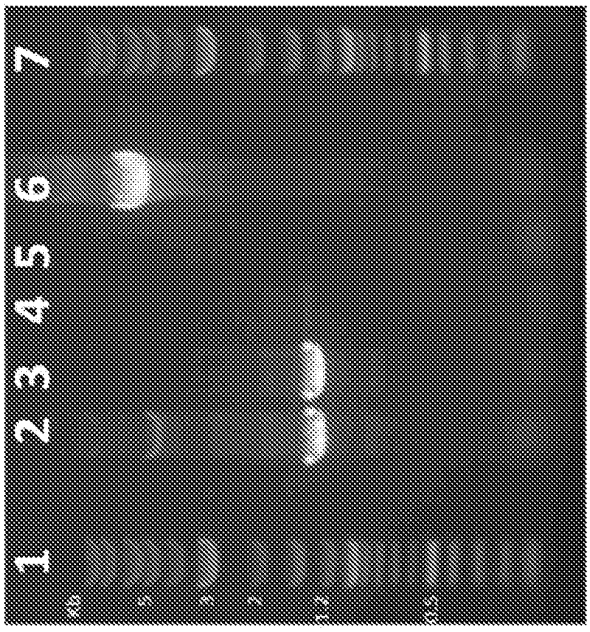


FIG. 25

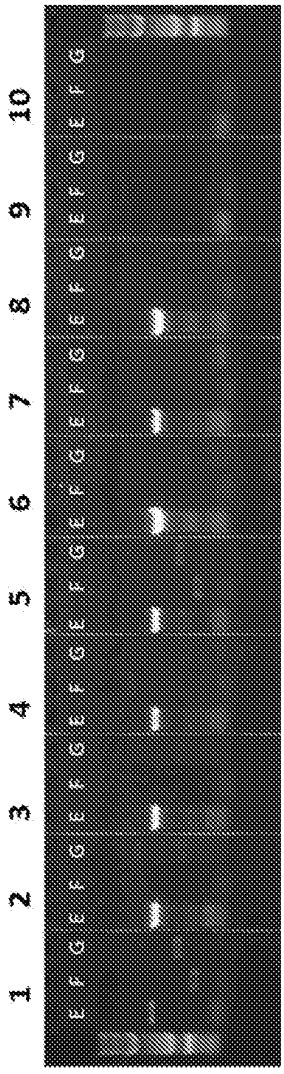
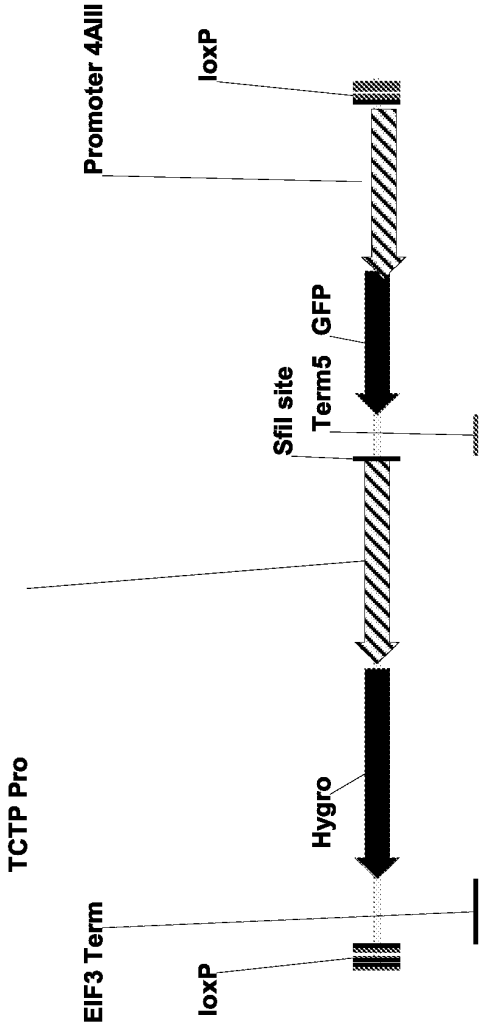


FIG. 26



loxP-HygR-GFP-loxP Cassette

FIG. 27

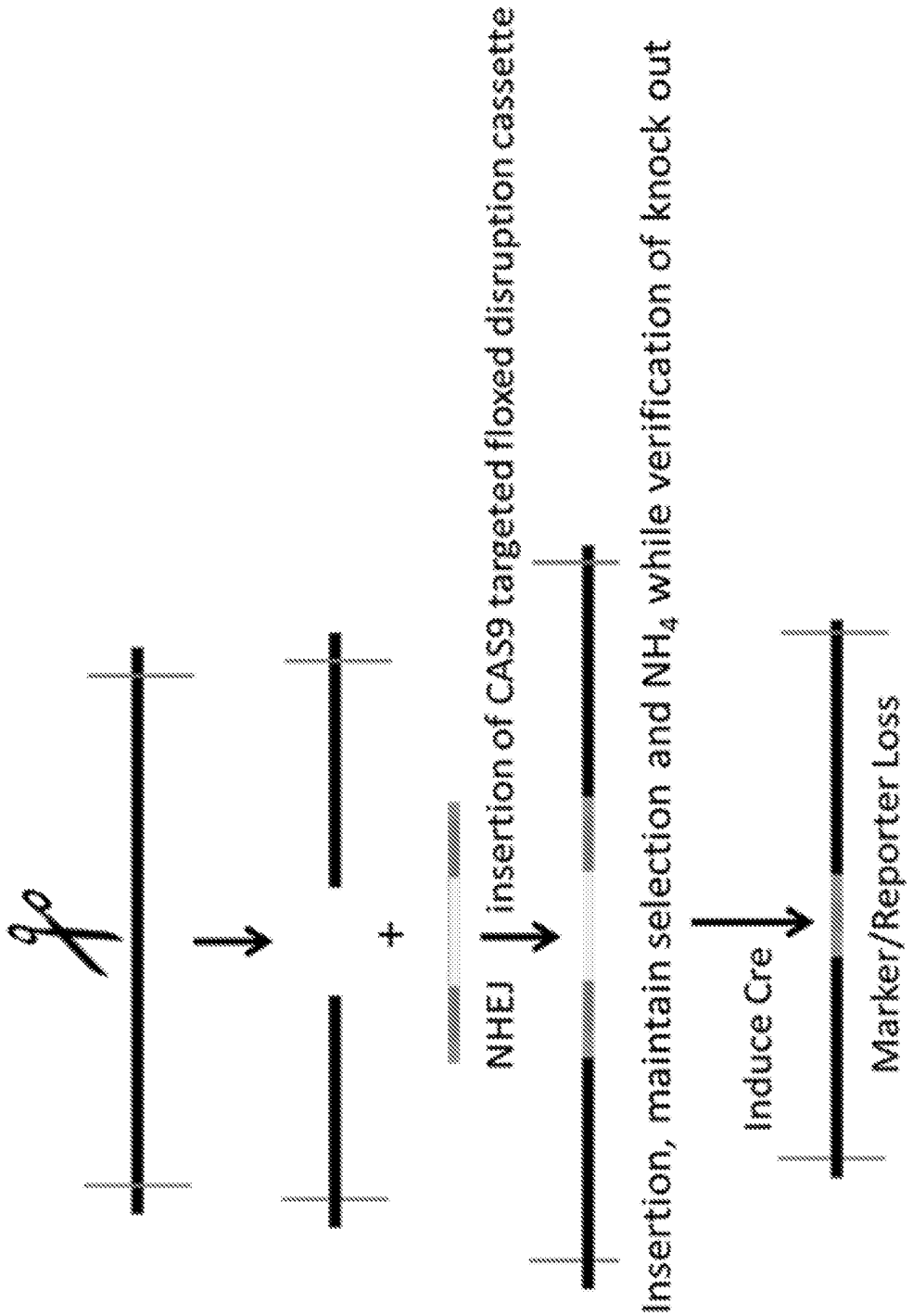

NHEJ
insertion of CAS9 targeted floxed disruption cassette
Insertion, maintain selection and NH4 while verification of knock out
Induce Cre
Marker/Reporter Loss

FIG. 28

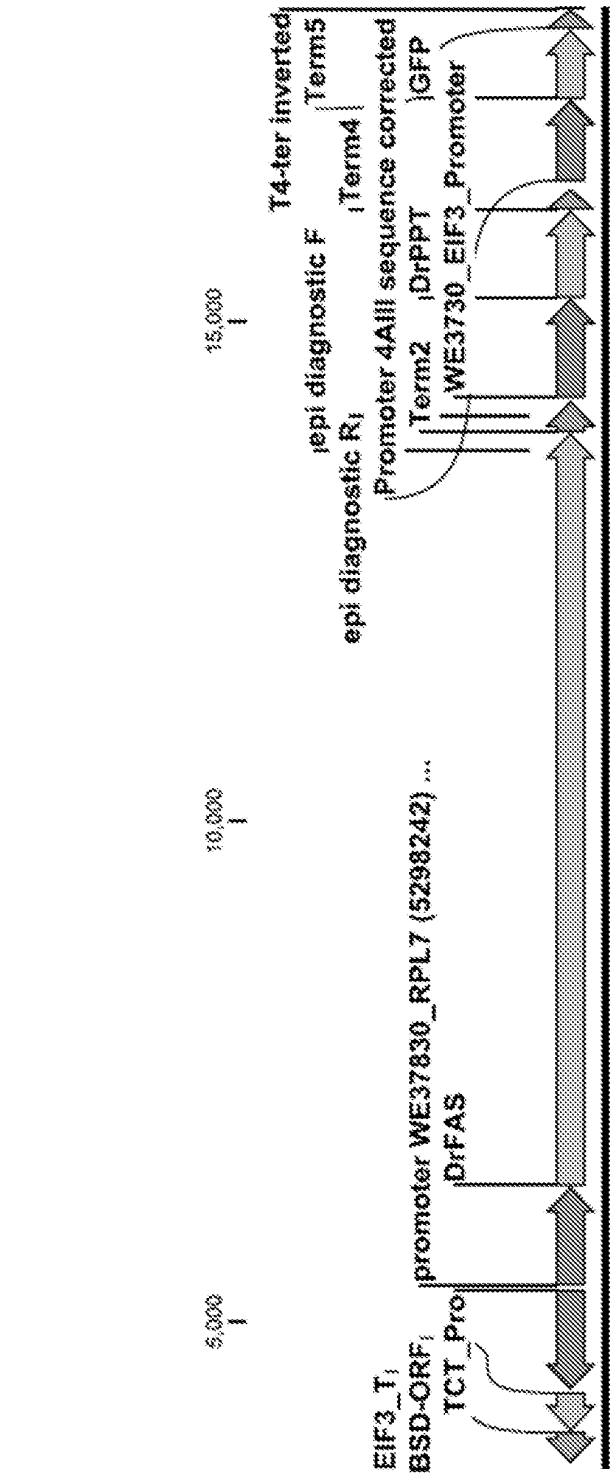

5,000
10,000
15,000
EIF3_T
BSD-ORF
TCT_Pro
promoter WE37830_RPL7 (5298242) ...
DrFAS
epi diagnostic R
epi diagnostic F
Promoter 4AIII sequence corrected
Term2
DrPPT
WE3730_EIF3_Promoter
GFP
T4-ter inverted
Term5
Term4

FIG. 29

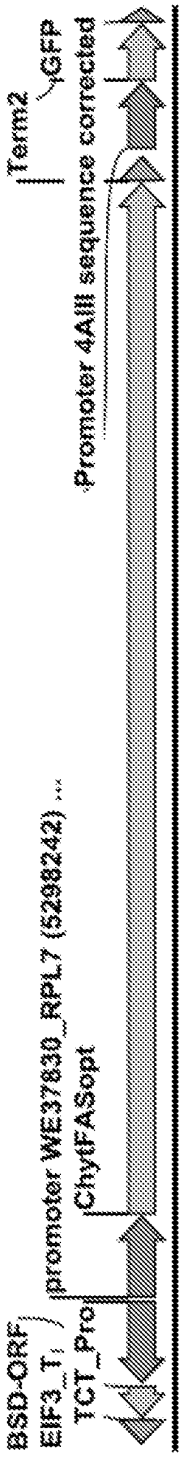

5,000
10,000
15,000
20,000
BSD-ORF
EIF3_T1
TCT_Pro
promoter WE37830_RPL7 (5298242) ...
ChytFASopt
Promoter 4Alll sequence corrected
GFP
Term2
Term5

FIG. 30

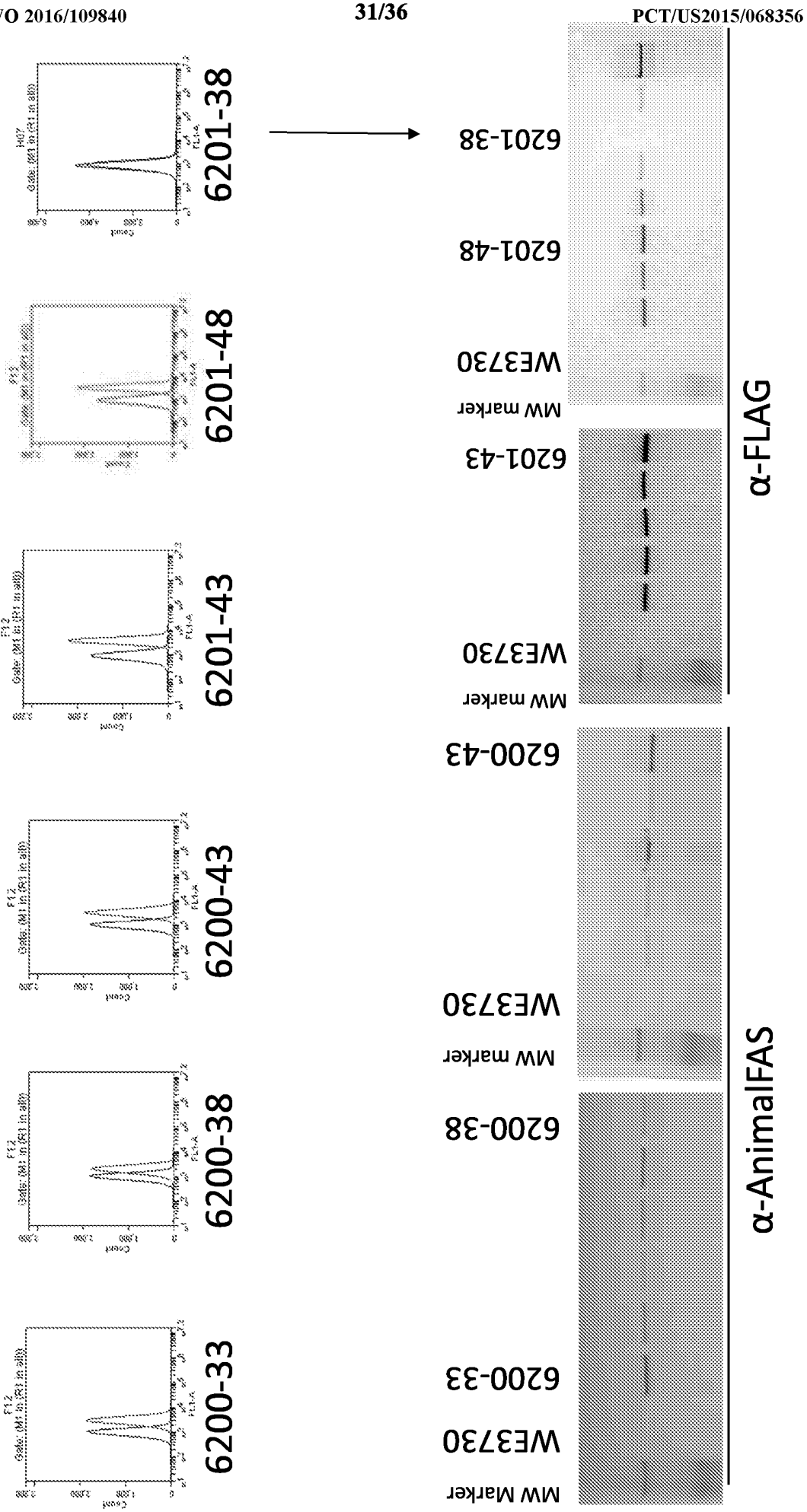

6200-33
6200-38
6200-43
6201-43
6201-48
6201-38
MW Marker
WE3730
α-AnimalFAS
α-FLAG

FIG. 31

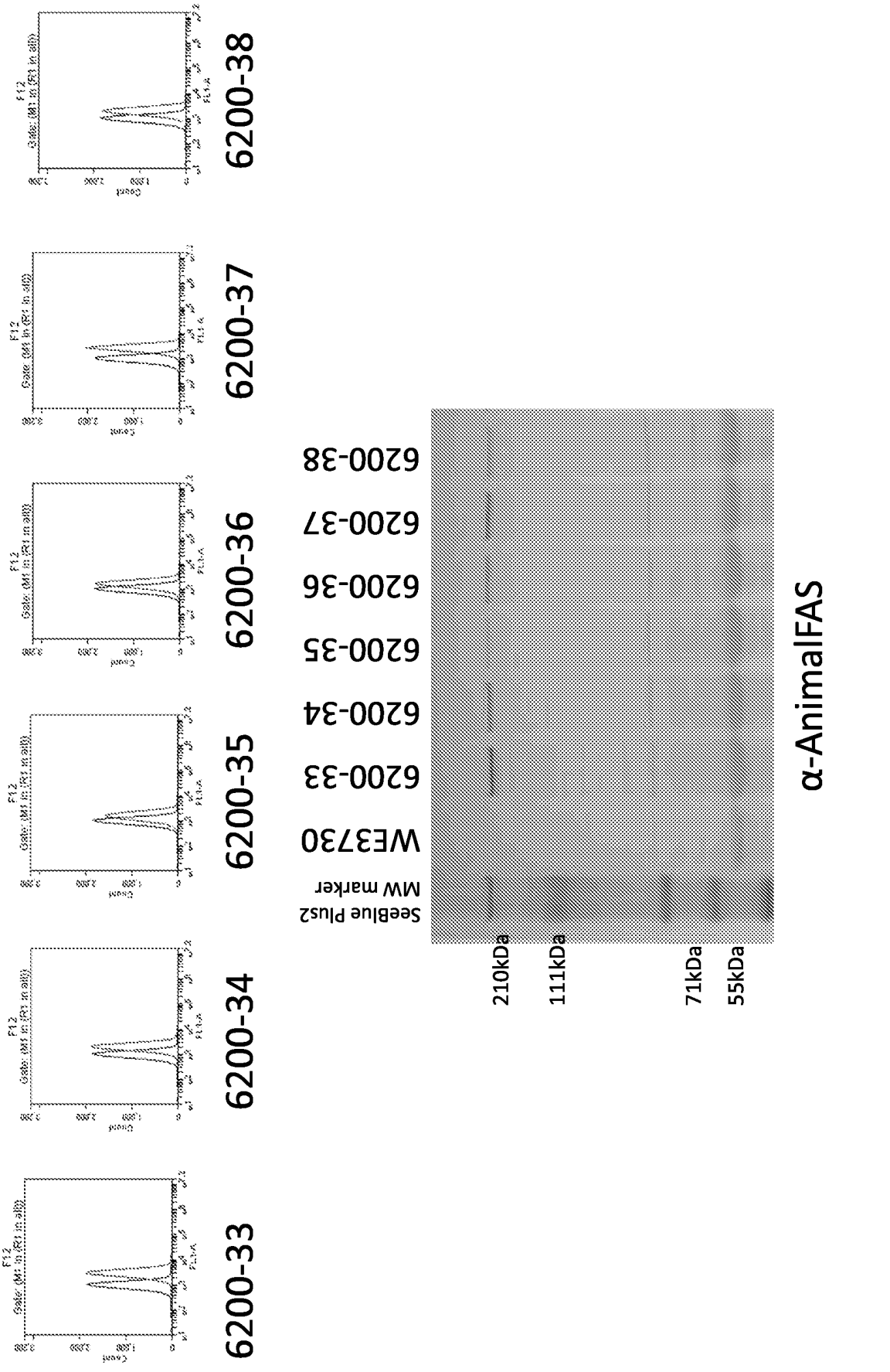
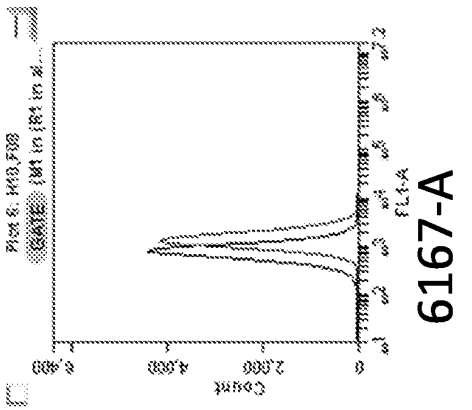
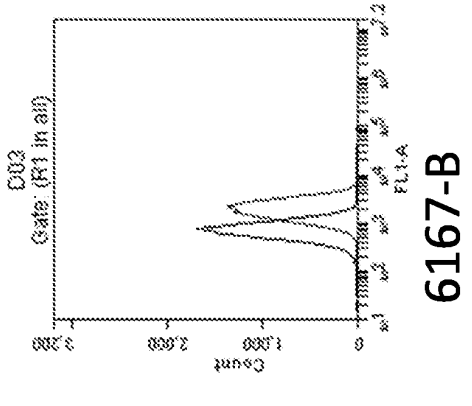

6200-33
6200-34
6200-35
6200-36
6200-37
6200-38
SeeBlue Plus2 MW marker
WE3730
6200-33
6200-34
6200-35
6200-36
6200-37
6200-38
210kDa
111kDa
71kDa
55kDa
α-AnimalFAS

FIG. 32


6167-A


6167-B

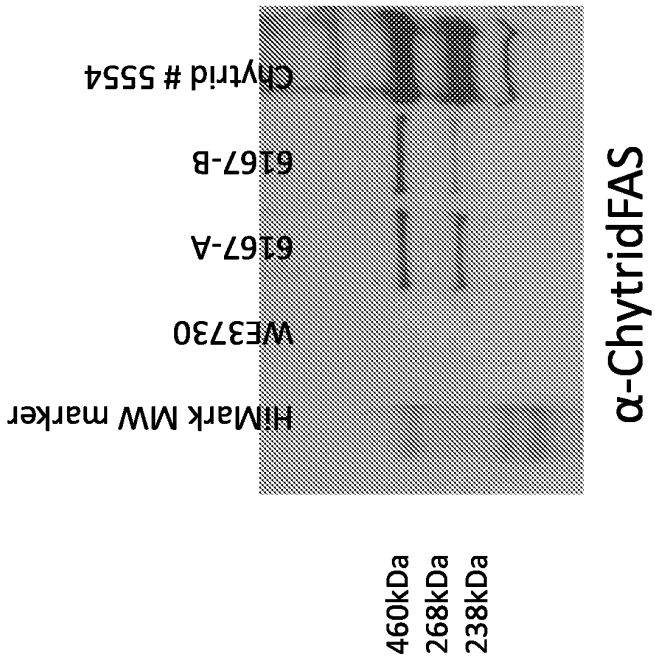

α-ChytridFAS
460kDa
268kDa
238kDa
HiMark MW marker
WE3730
6167-A
6167-B
Chytrid # 5554

FIG. 33

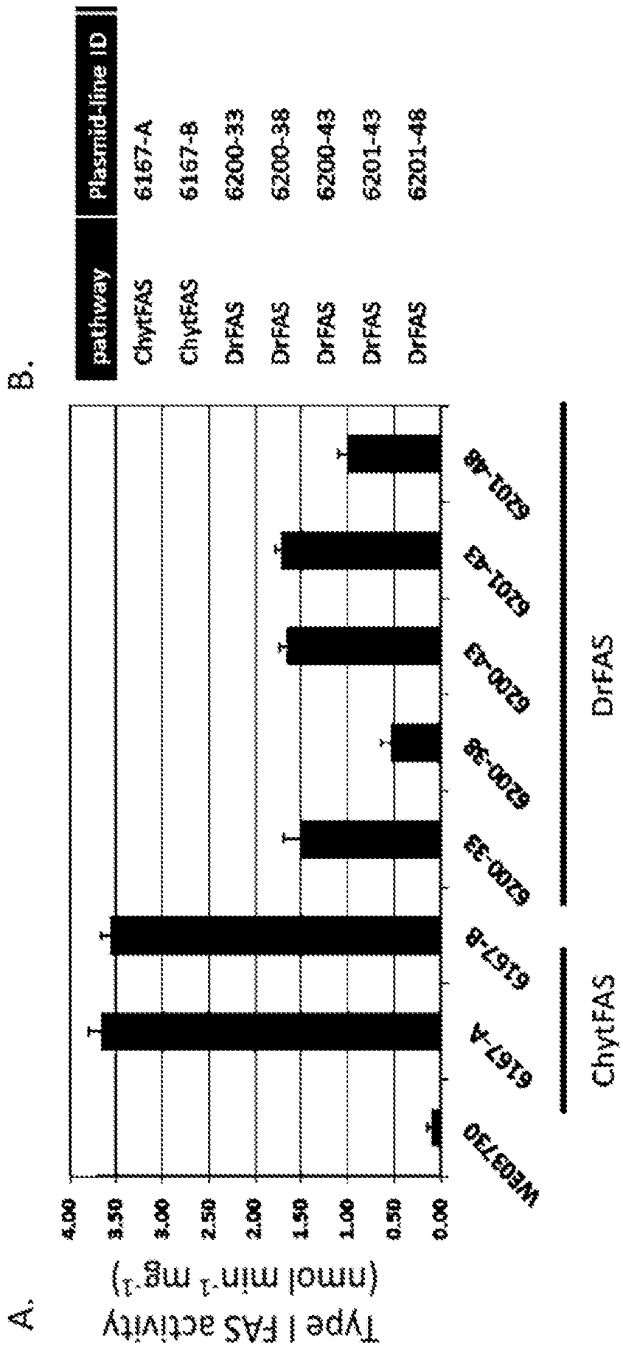

A.
Type I FAS activity
(nmol min-1 mg-1)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
WEB3730
6167-A
6167-B
6200-33
6200-38
6200-43
6201-43
6201-48
ChytFAS
DrFAS
B.
pathway
Plasmid-line ID
ChytFAS 6167-A
ChytFAS 6167-B
DrFAS 6200-33
DrFAS 6200-38
DrFAS 6200-43
DrFAS 6201-43
DrFAS 6201-48

FIG. 34

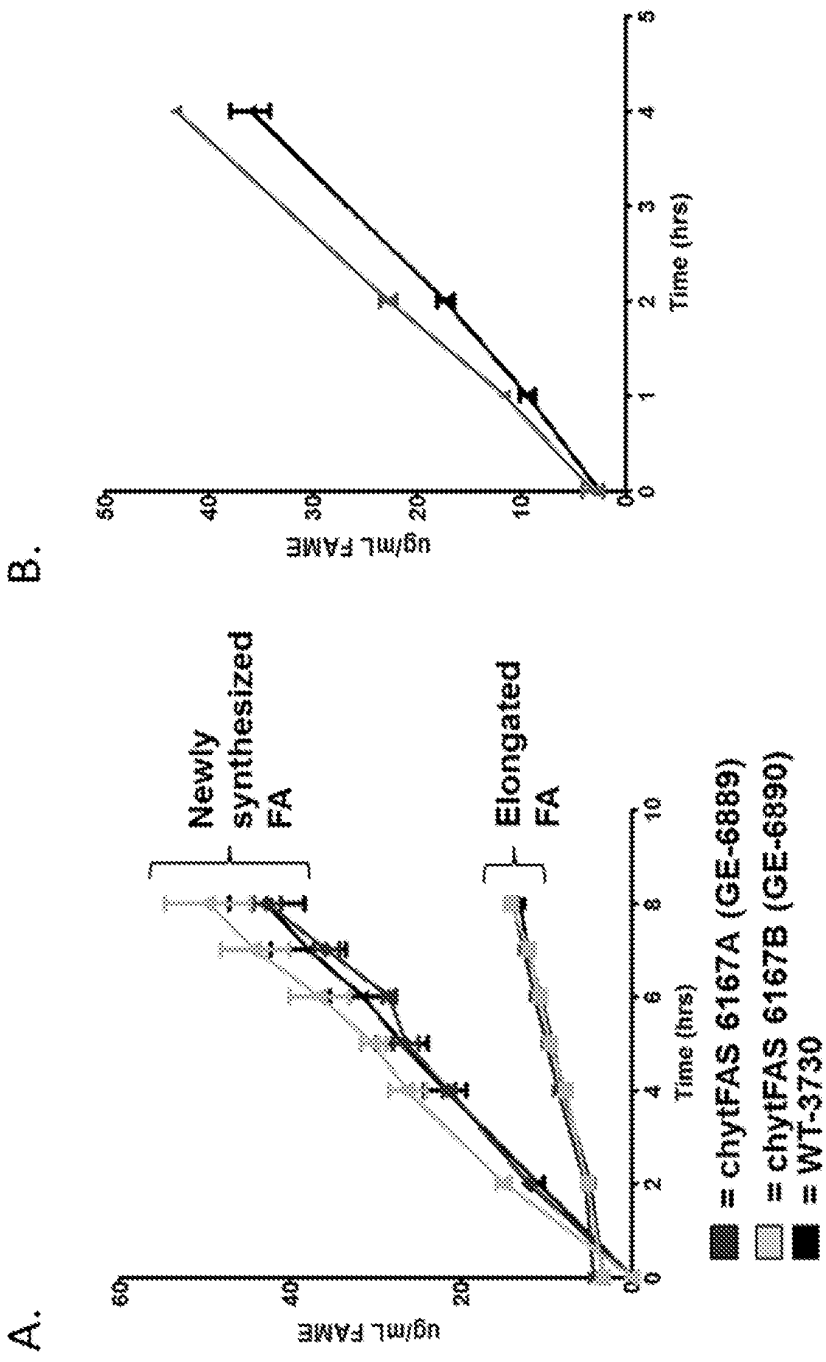
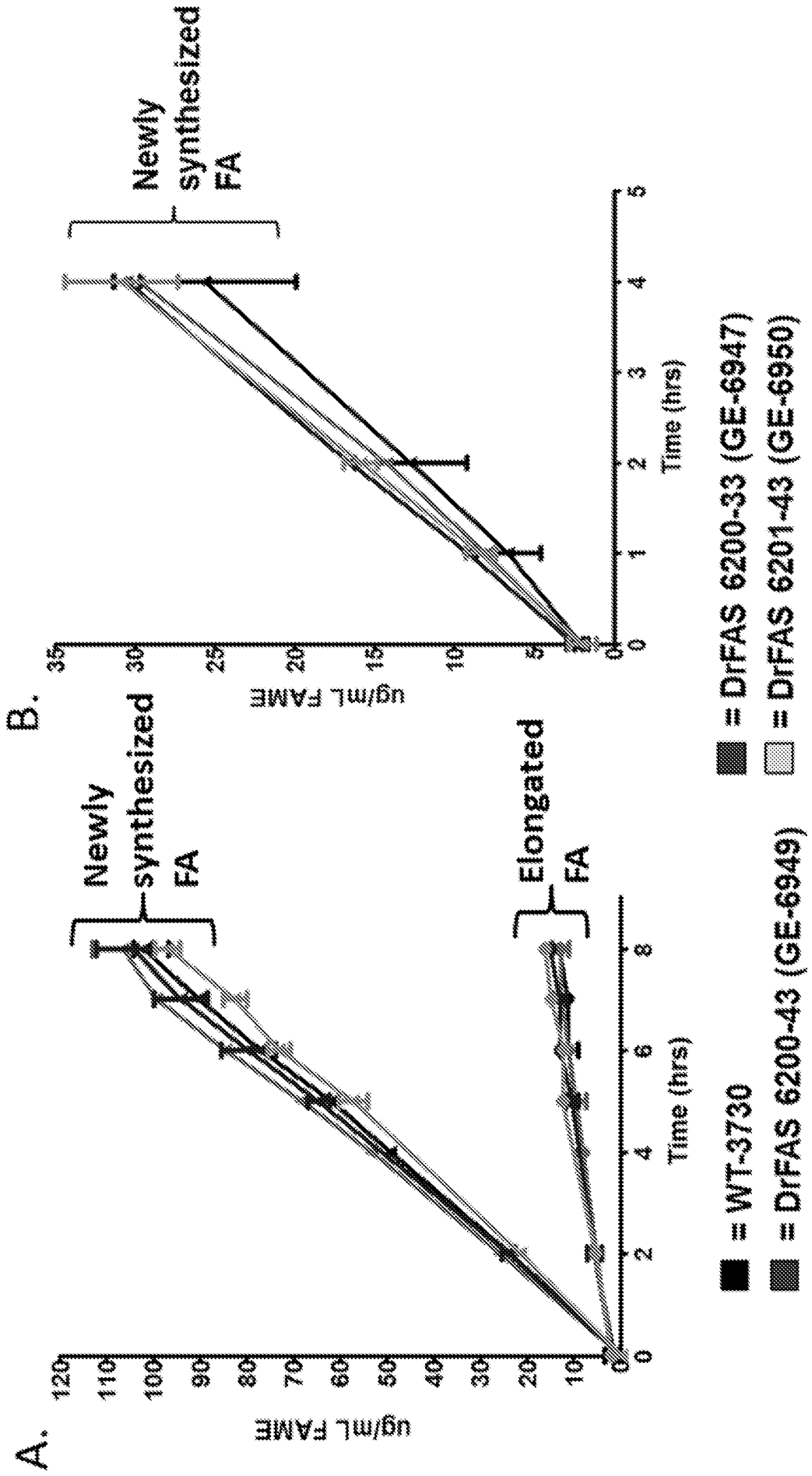

A.
B.
ug/mL FAME
Time (hrs)
Newly synthesized FA
Elongated FA
= chytFAS 6167A (GE-6889)
= chytFAS 6167B (GE-6890)
= WT-3730

FIG. 35


A.
B.
ug/mL FAME
Time (hrs)
Newly synthesized FA
Elongated FA
= WT-3730
= DrFAS 6200-43 (GE-6949)
= DrFAS 6200-33 (GE-6947)
= DrFAS 6201-43 (GE-6950)

FIGS. 36A-36B