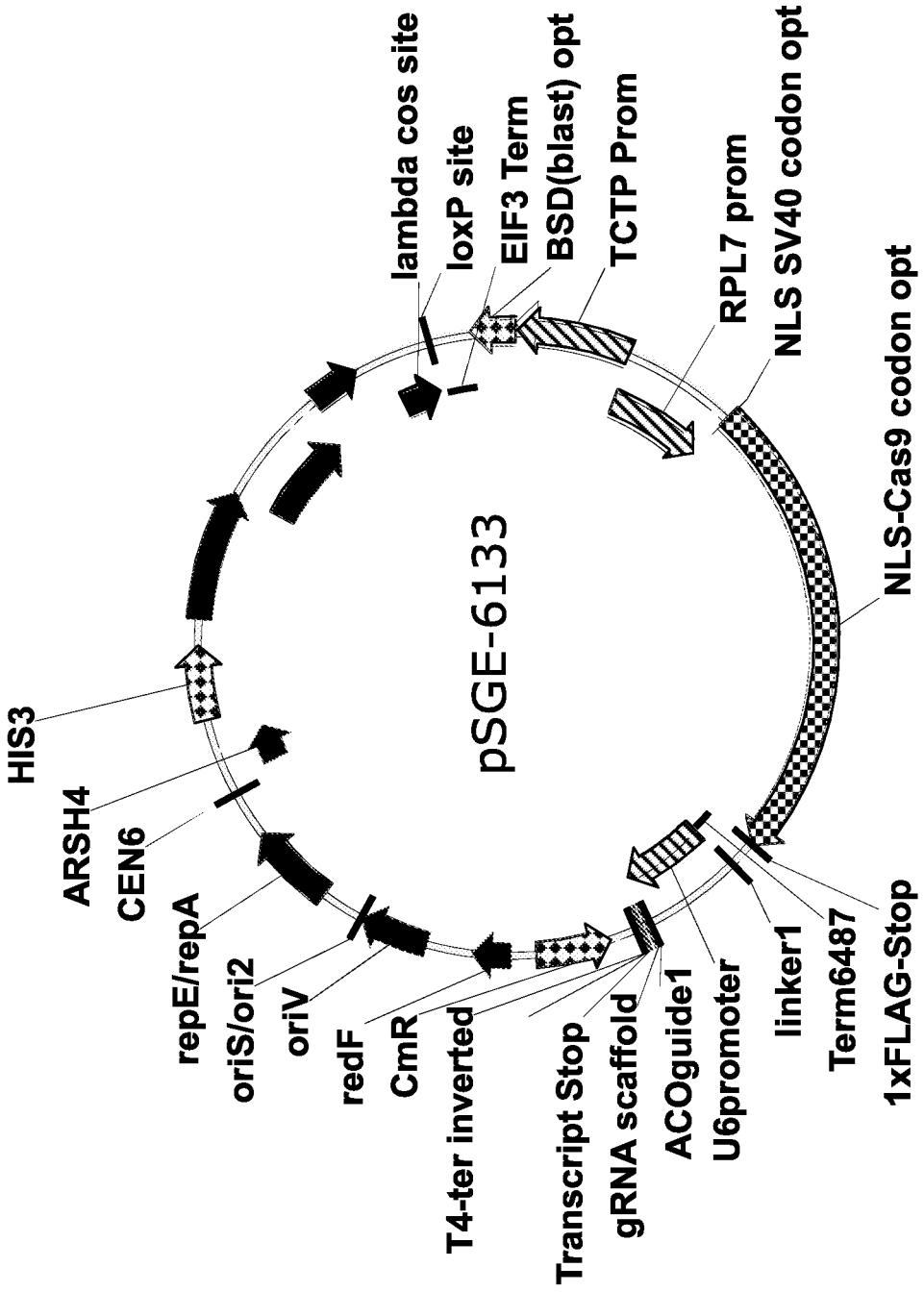

pSGE-6133
lambda cos site
loxP site
EIF3 Term
BSD(blast) opt
TCTP Prom
RPL7 prom
NLS SV40 codon opt
NLS-Cas9 codon opt
1xFLAG-Stop
Term6487
linker1
U6promoter
ACOguide1
gRNA scaffold
Transcript Stop
T4-ter inverted
CmR
redF
oriV
oriS/ori2
repE/repA
CEN6
ARSH4
HIS3

FIG. 1

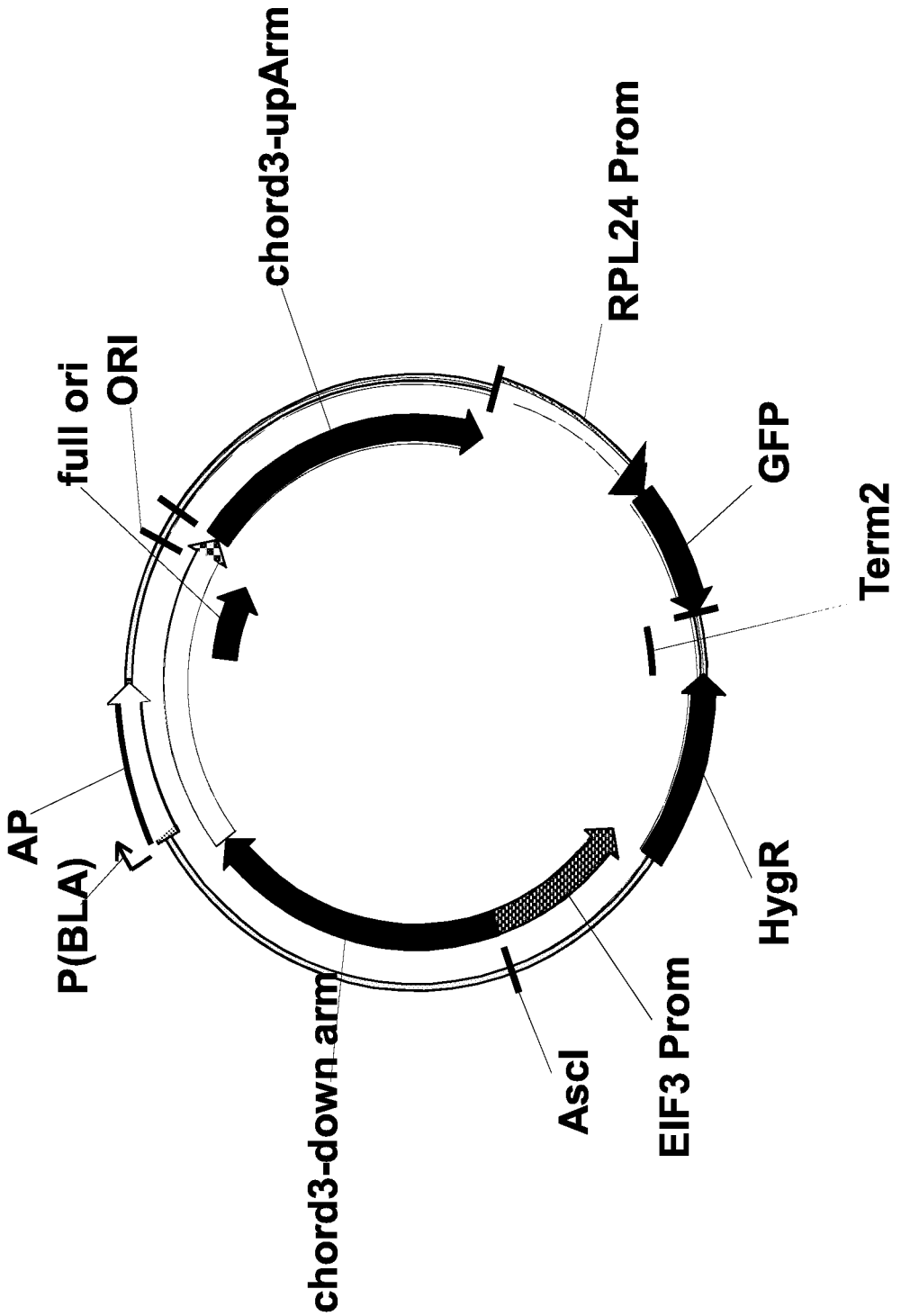

chord3-upArm
RPL24 Prom
GFP
Term2
HygR
EIF3 Prom
AscI
chord3-down arm
P(BLA)
AP
full ori
ORI

FIG. 2

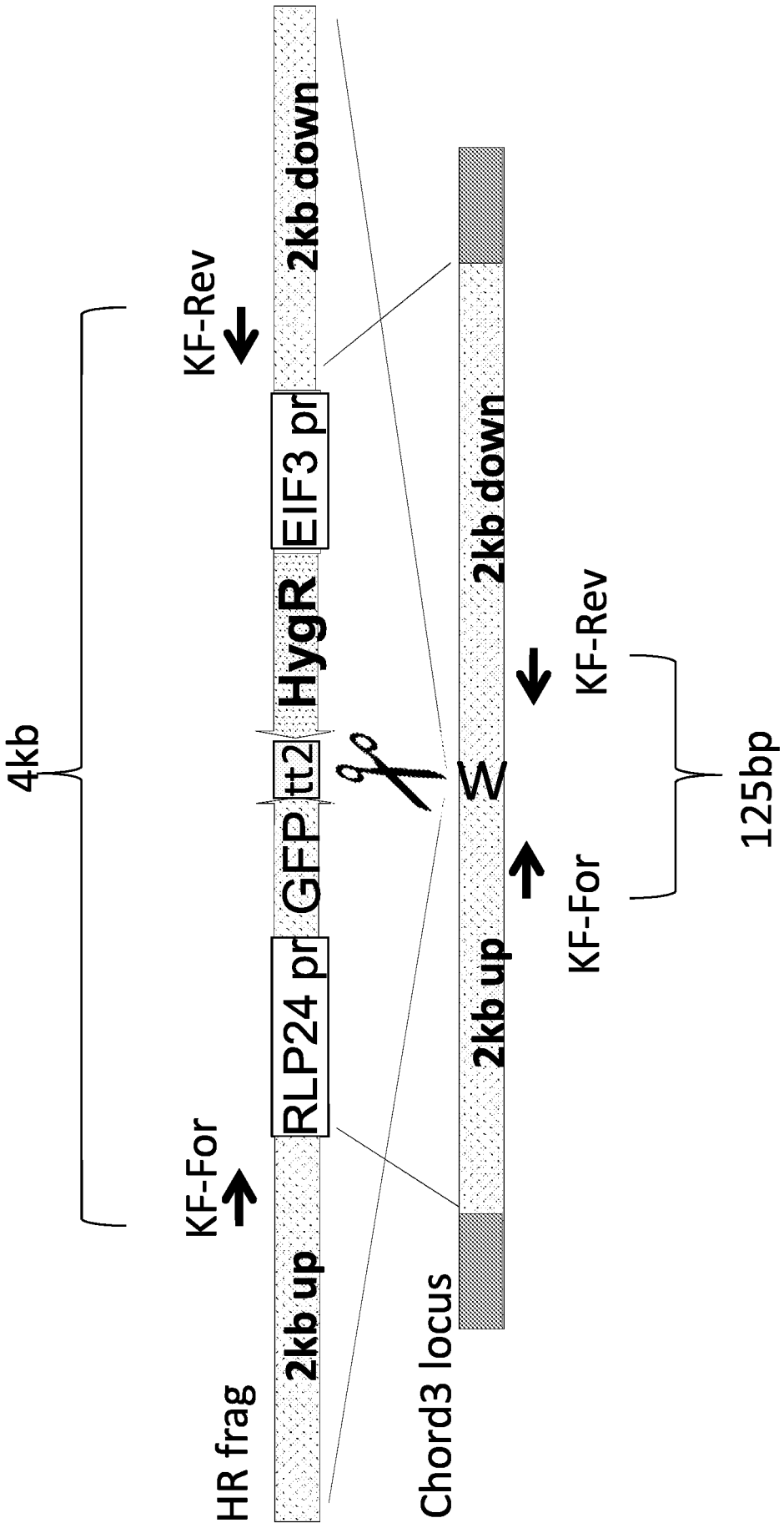

HR frag
2kb up
RLP24 pr
GFP
tt2
HygR
EIF3 pr
2kb down
KF-For
KF-Rev
4kb
Chord3 locus
2kb up
2kb down
KF-For
KF-Rev
125bp

FIG. 3

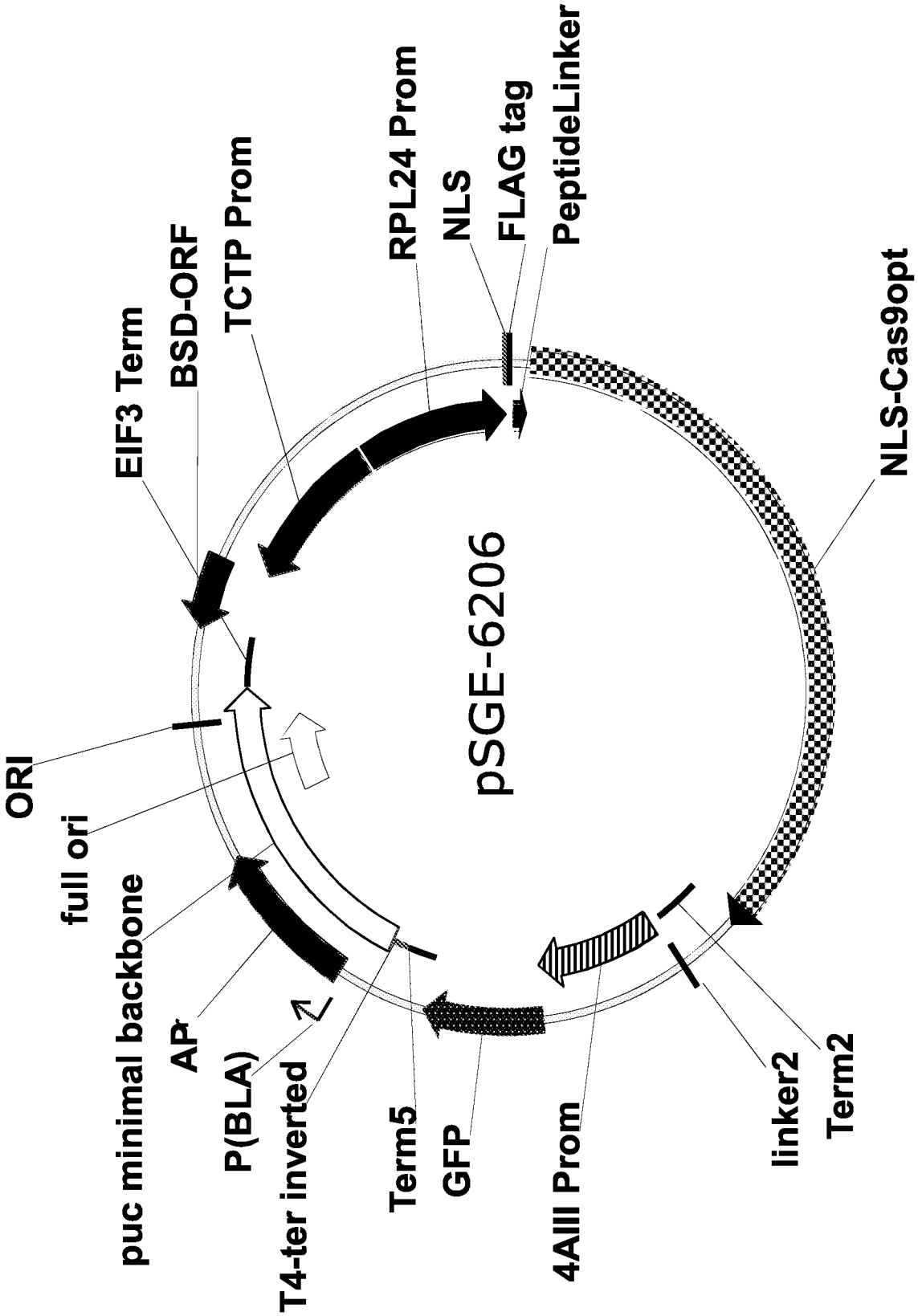
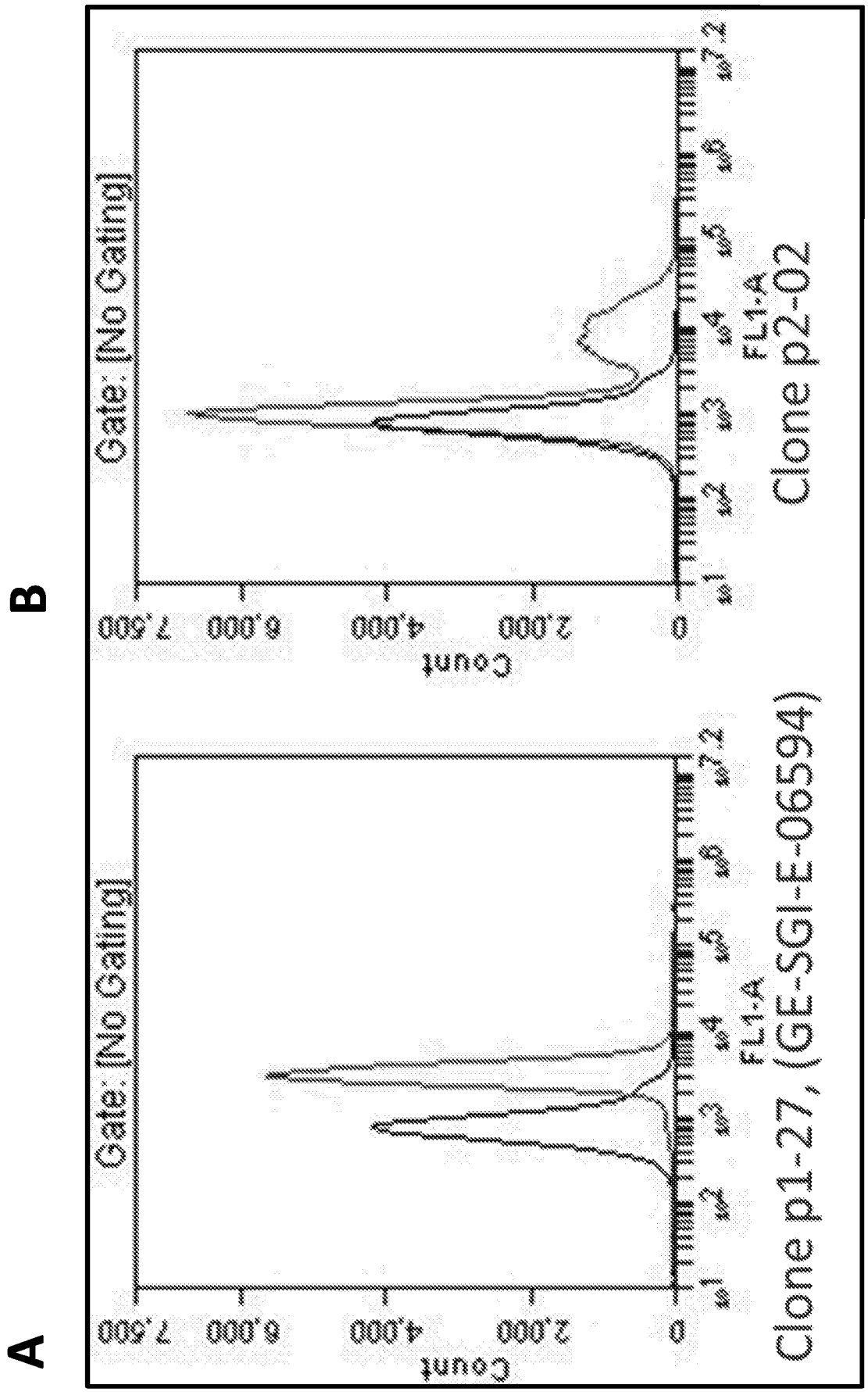

pSGE-6206
NLS-Cas9opt
PeptideLinker
FLAG tag
NLS
RPL24 Prom
TCTP Prom
BSD-ORF
EIF3 Term
ORI
full ori
puc minimal backbone
AP
P(BLA)
T4-ter inverted
Term5
GFP
4AIII Prom
linker2
Term2

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

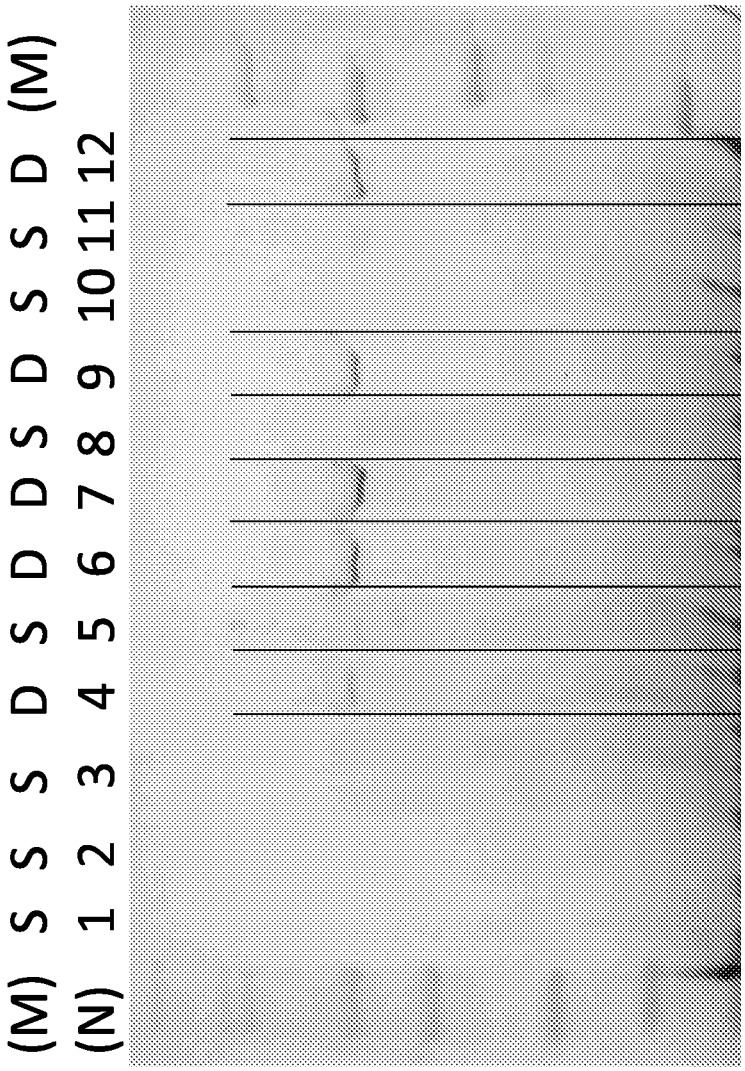


FIG. 6

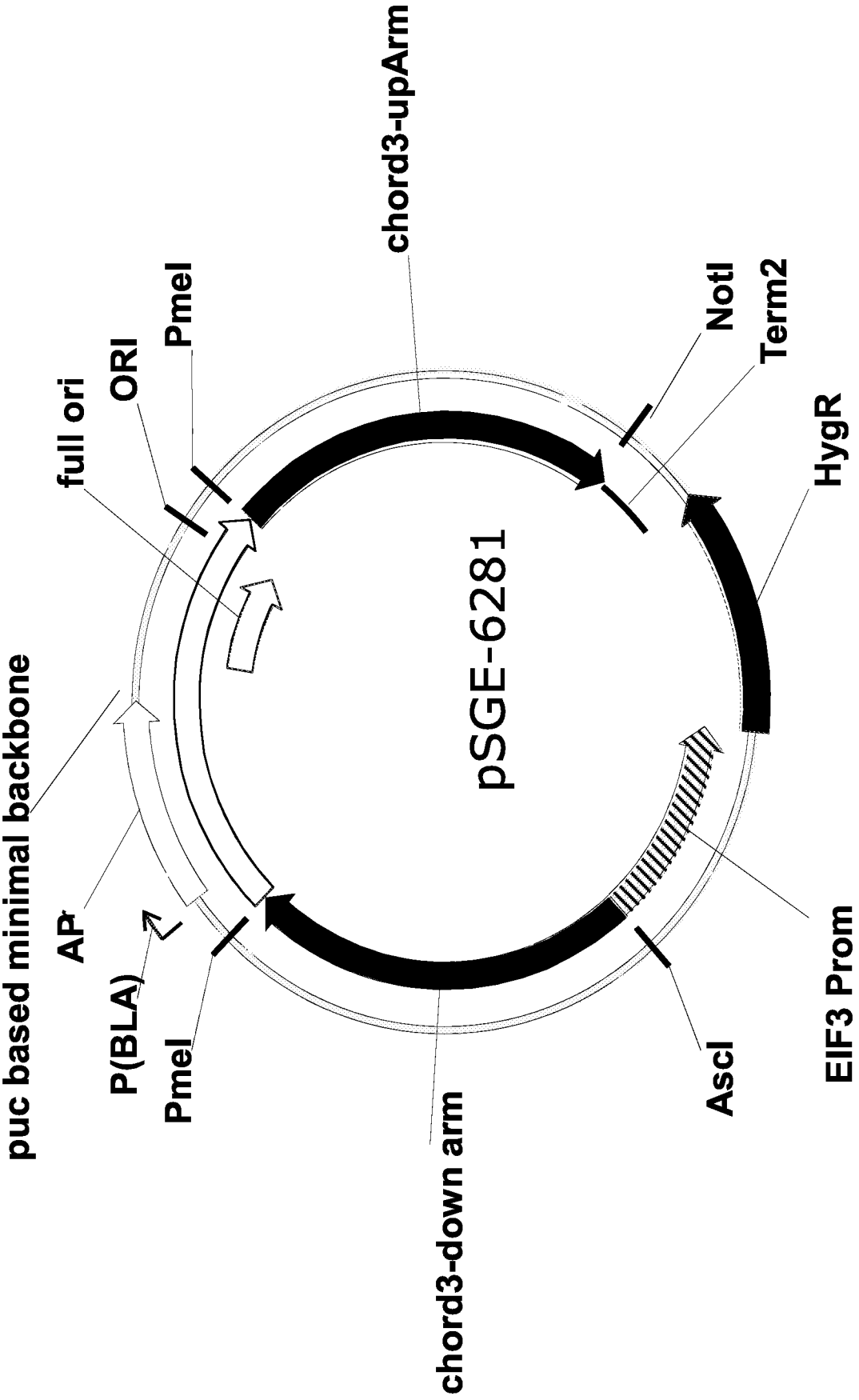

pSGE-6281
chord3-upArm
NotI
Term2
HygR
EIF3 Prom
AscI
chord3-down arm
PmeI
P(BLA)
AP
puc based minimal backbone
full ori
ORI
PmeI

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

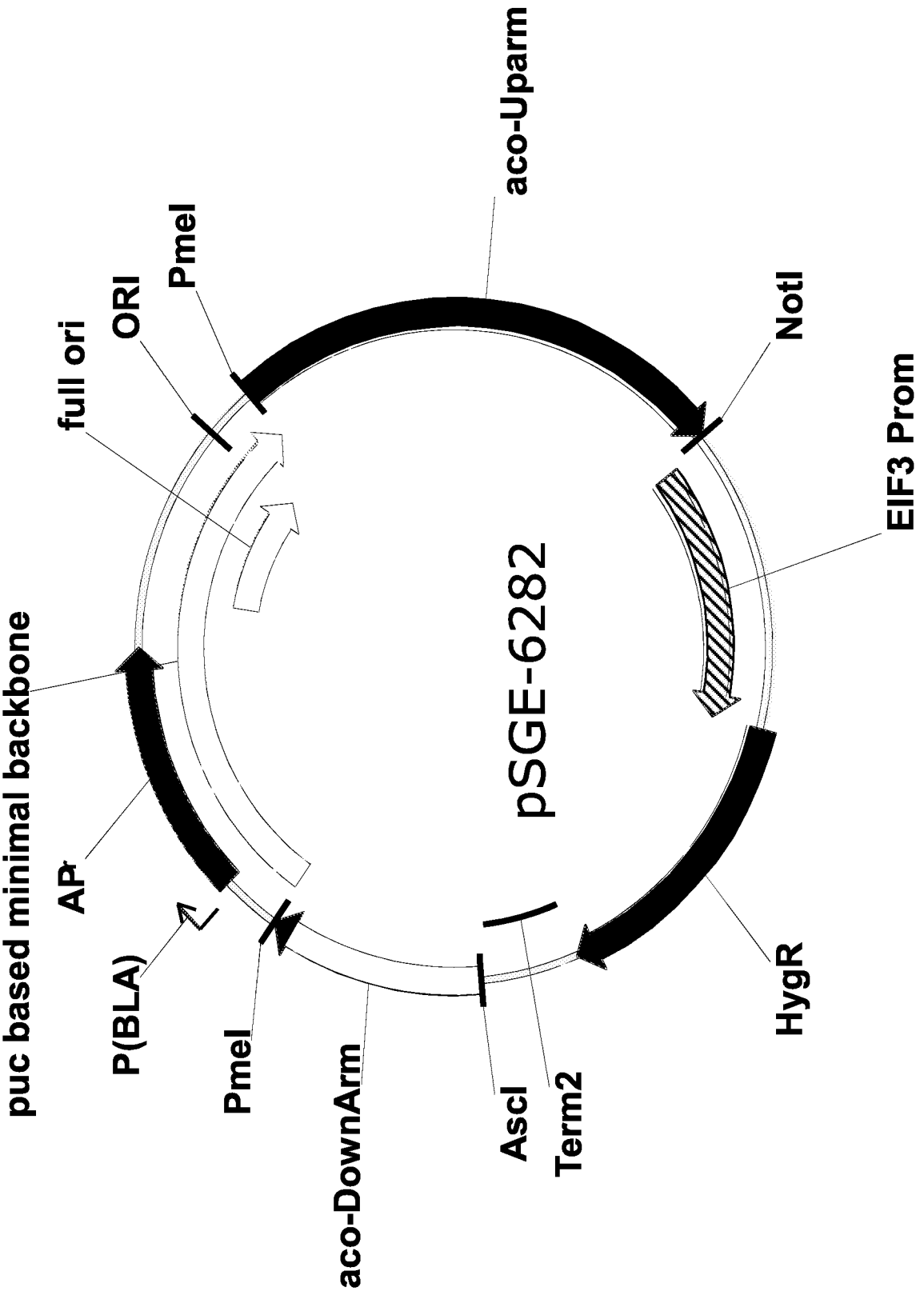

pSGE-6282
aco-Uparm
NotI
EIF3 Prom
HygR
Term2
AscI
aco-DownArm
PmeI
P(BLA)
APr
puc based minimal backbone
full ori
ORI
PmeI

FIG. 9

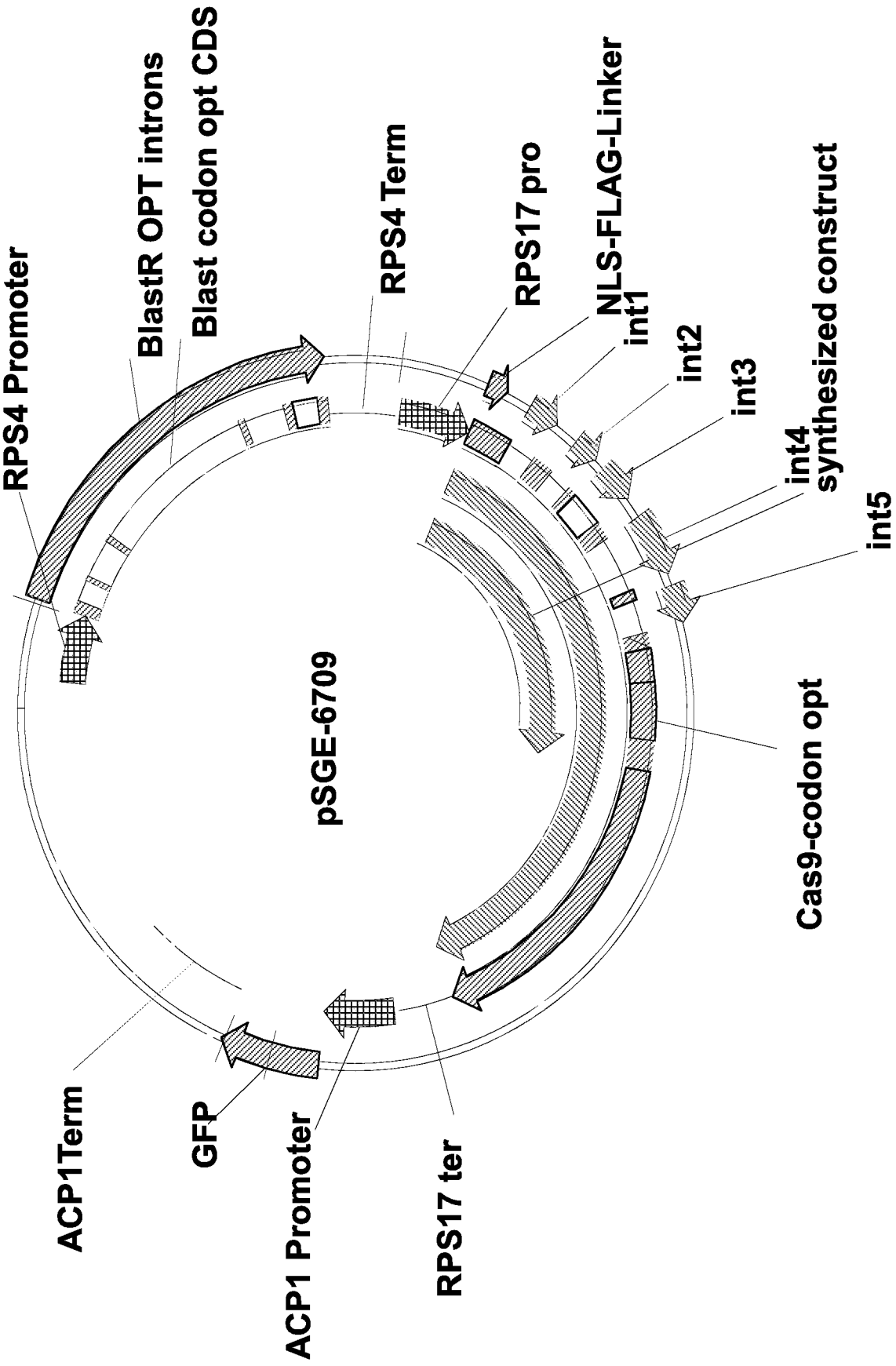

RPS4 Promoter
BlastR OPT introns
Blast codon opt CDS
RPS4 Term
RPS17 pro
NLS-FLAG-Linker
int1
int2
int3
int4
synthesized construct
int5
Cas9-codon opt
pSGE-6709
ACP1Term
GFP
ACP1 Promoter
RPS17 ter

FIG. 10

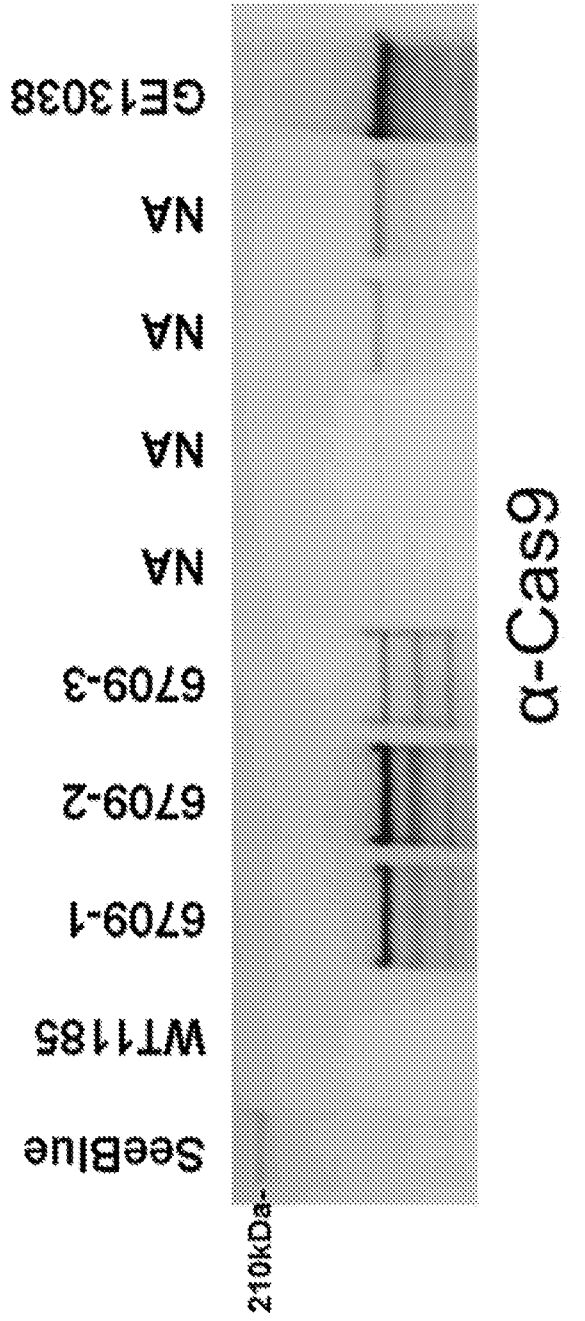

SeeBlue
WT1185
6709-1
6709-2
6709-3
NA
NA
NA
NA
GE13038
210kDa-
α-Cas9

FIG. 11

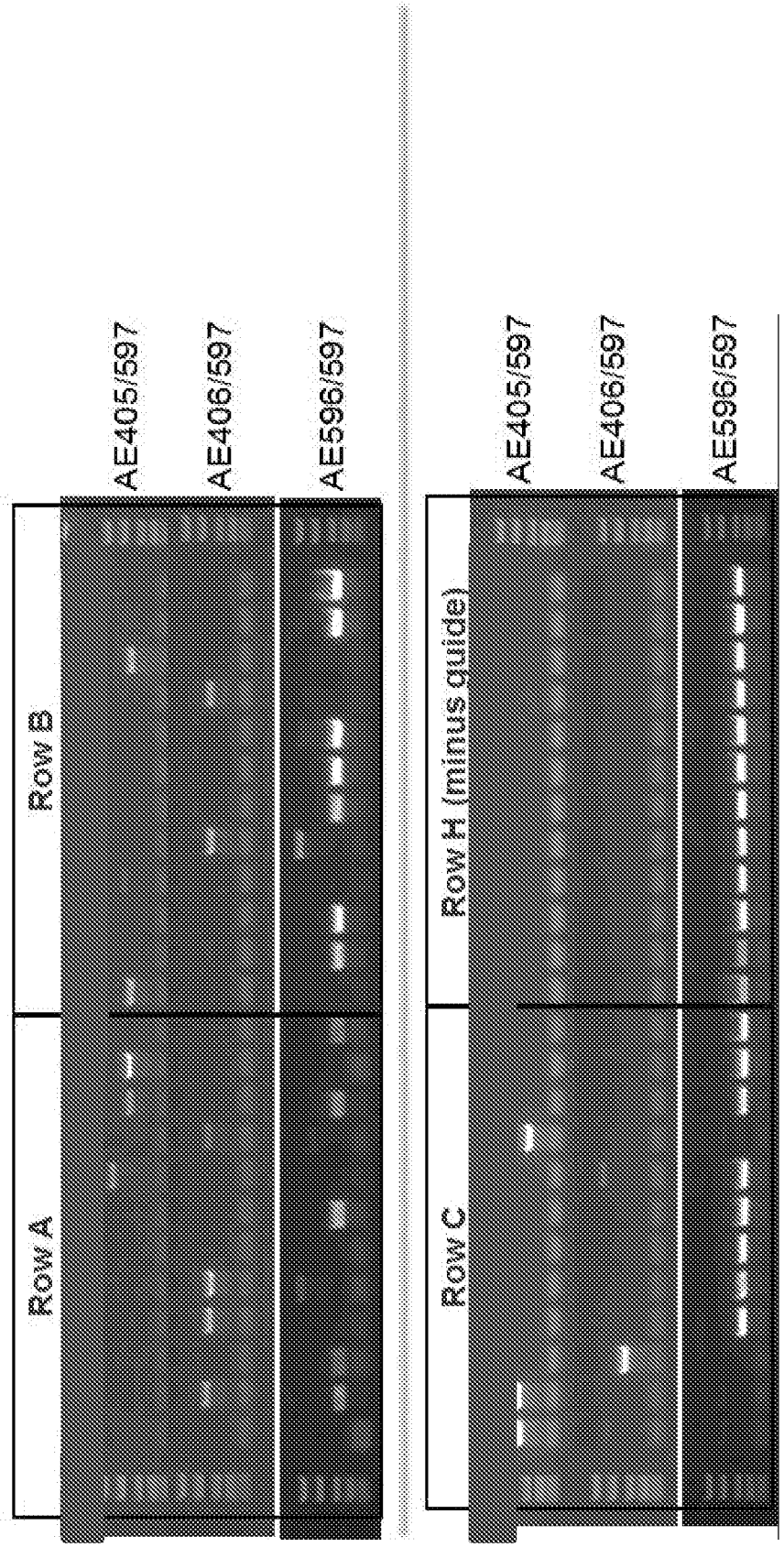
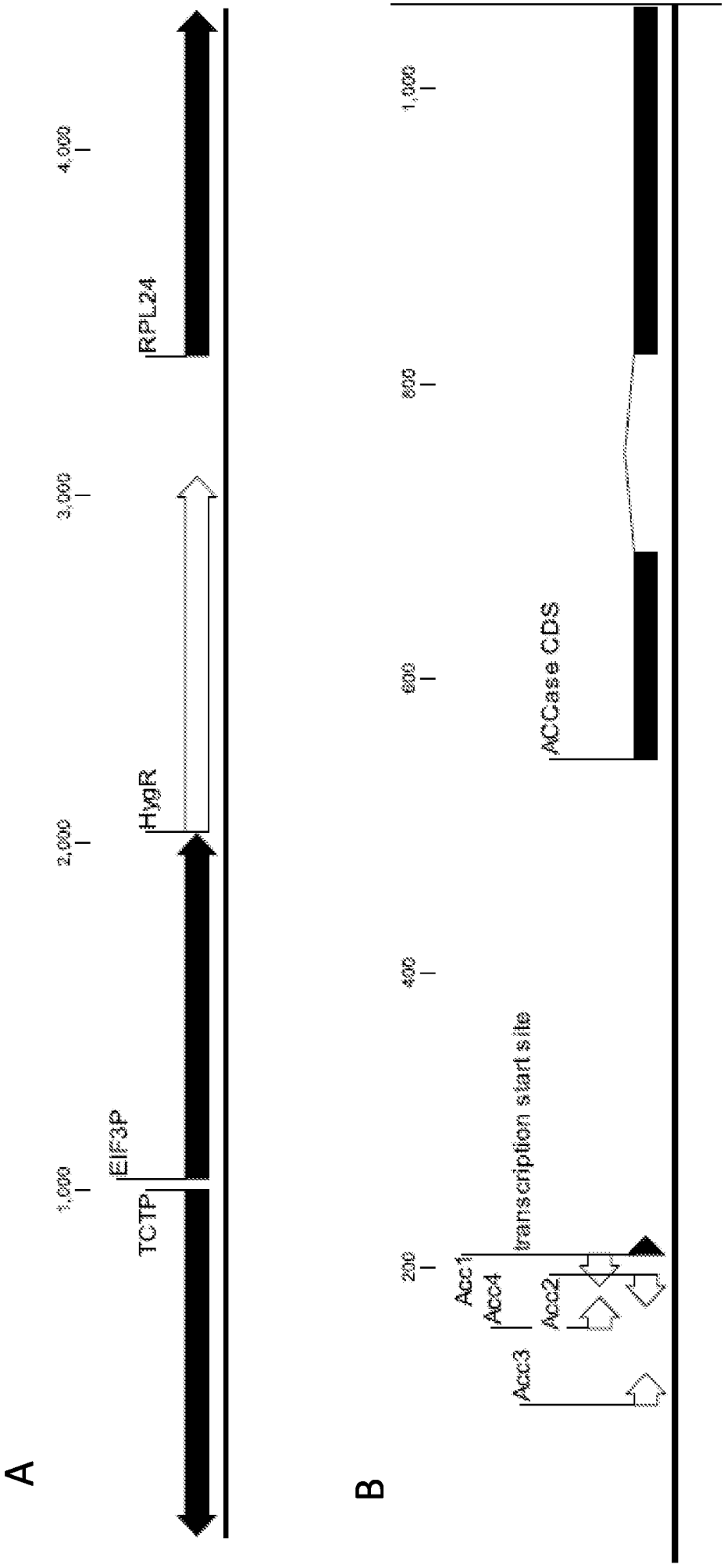
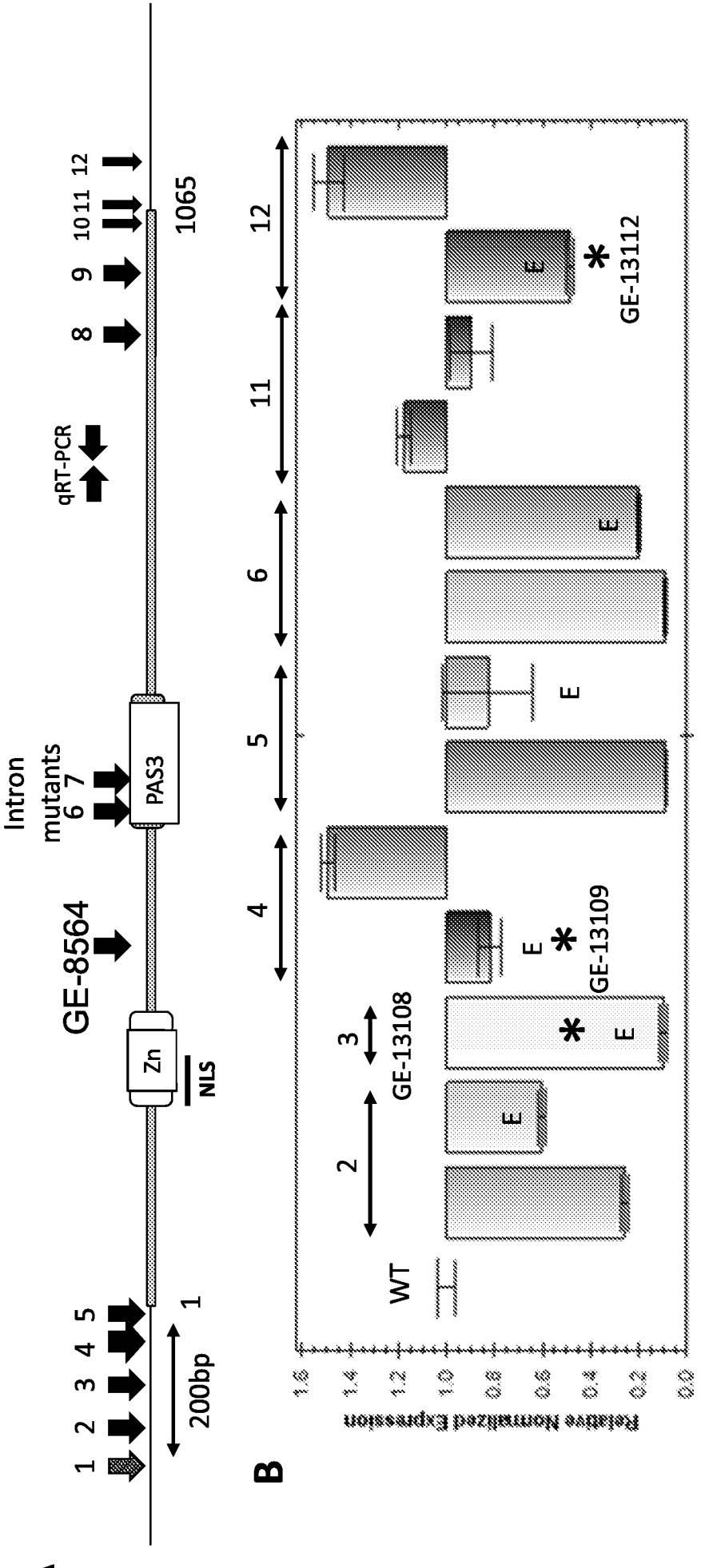

Row A
Row B
Row C
Row H (minus guide)
AE405/597
AE406/597
AE596/597
AE405/597
AE406/597
AE596/597

FIG. 12


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

FIGS. 13A-13B


A
1 2 3 4 5
200bp
1
1065
NLS
Zn
GE-8564
Intron mutants 6 7
PAS3
qRT-PCR
8 9 10 11 12
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
12
E
GE-13112

FIGS. 14A-14B

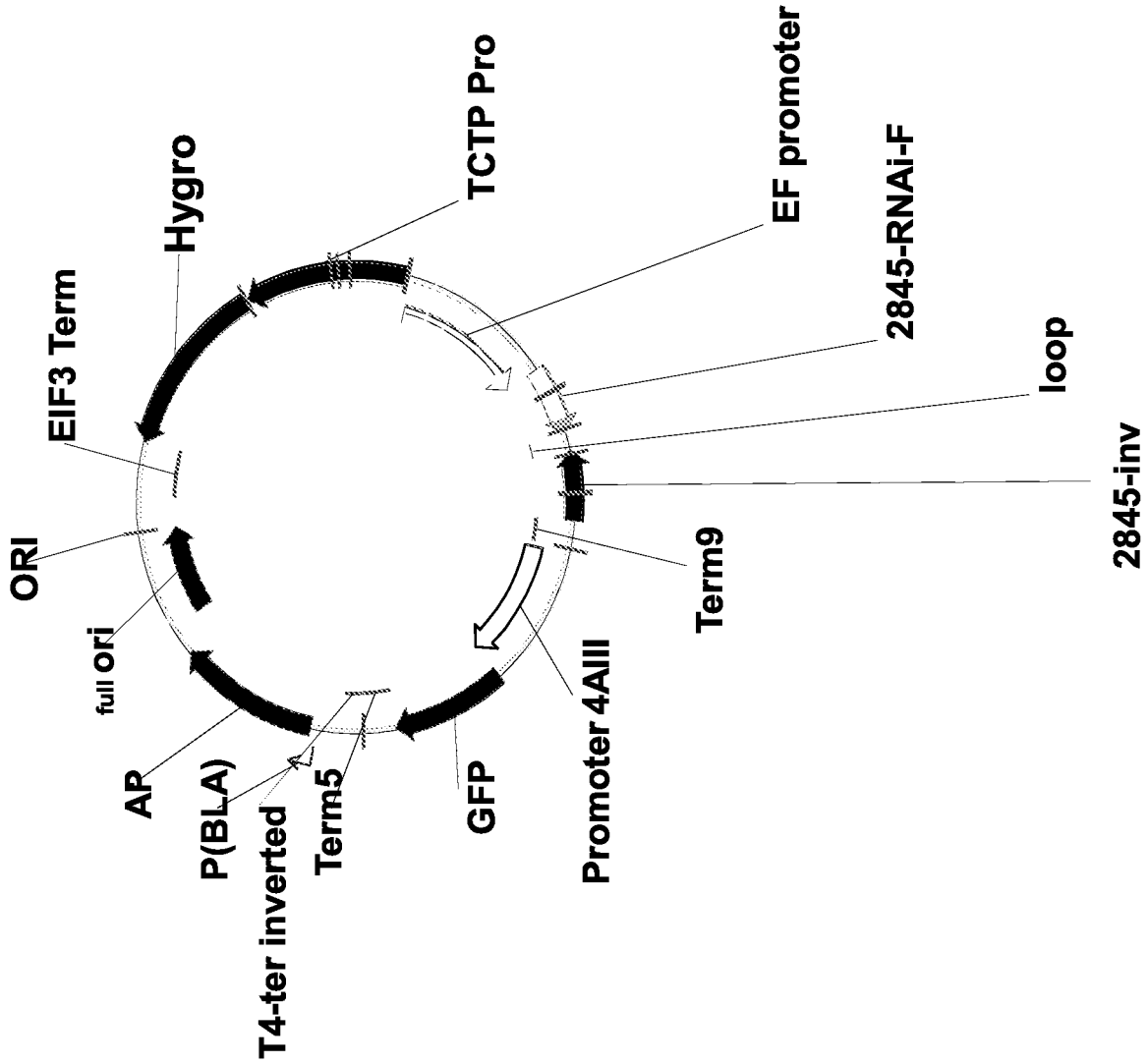

Hygro
TCTP Pro
EF promoter
2845-RNAi-F
loop
2845-inv
Term9
Promoter 4AIII
GFP
Term5
T4-ter inverted
P(BLA)
AP
full ori
ORI
EIF3 Term

FIG. 15

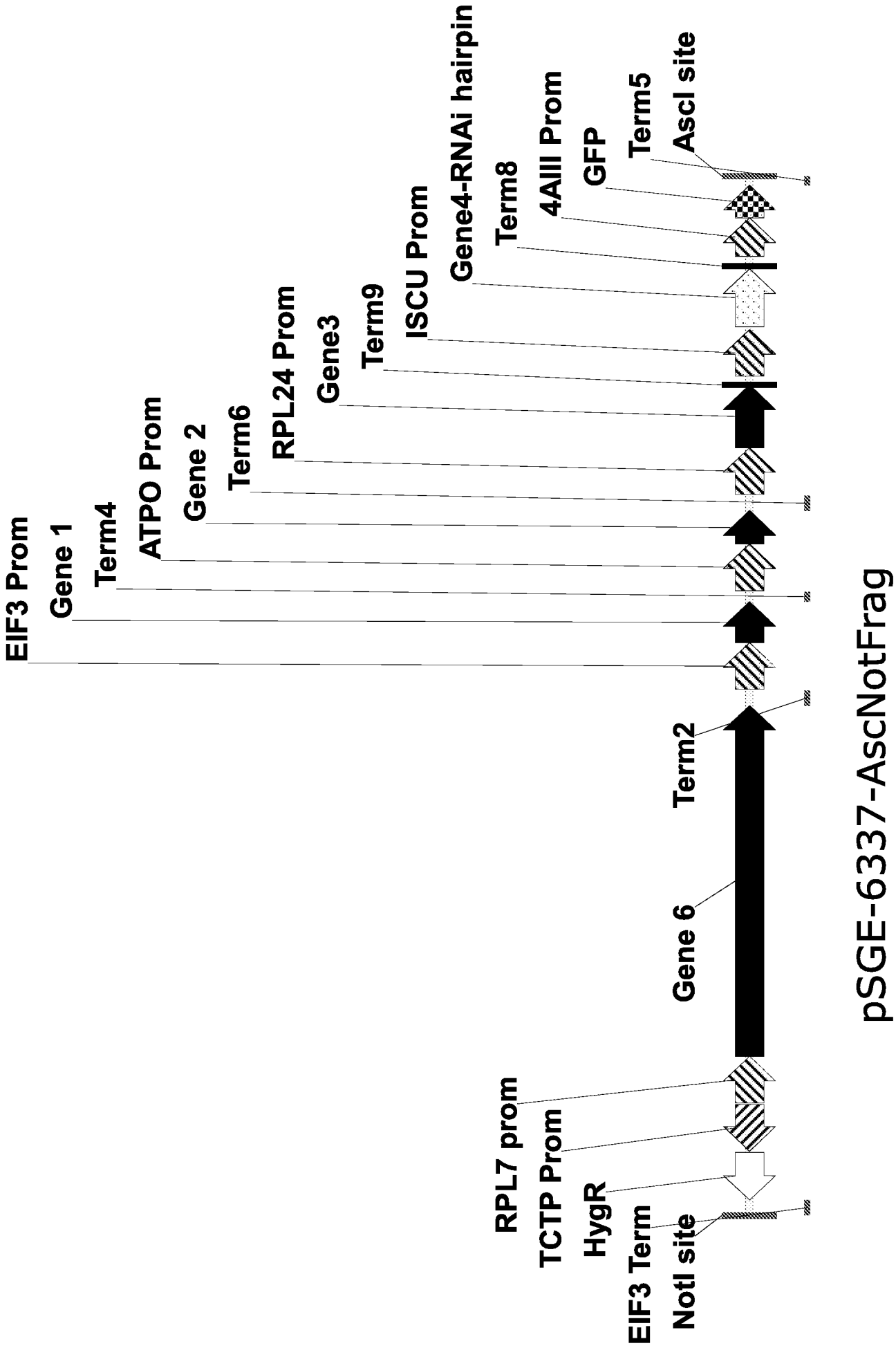

EIF3 Prom
Gene 1
Term4
ATPO Prom
Gene 2
Term6
RPL24 Prom
Gene3
Term9
ISCU Prom
Gene4-RNAi hairpin
Term8
4AIII Prom
GFP
Term5
AscI site
Term2
Gene 6
RPL7 prom
TCTP Prom
HygR
EIF3 Term
NotI site
pSGE-6337-AscNotFrag

FIG. 16

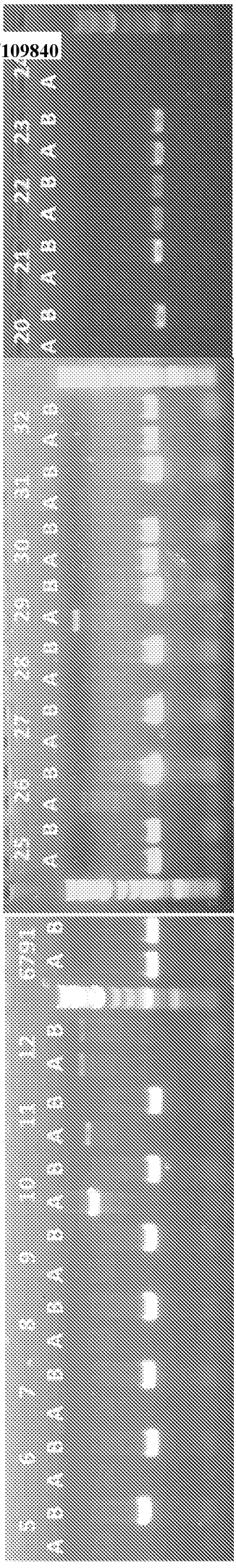

5 6 7 8 9 10 11 12 STD
A B A B A B A B A B A B A B A B A
20 21 22 23 24
A B A B A B A B A
23 24 25 26 27 28 29 30 31 32
A B A B A B A B A B A B A B A B A B

FIG. 17

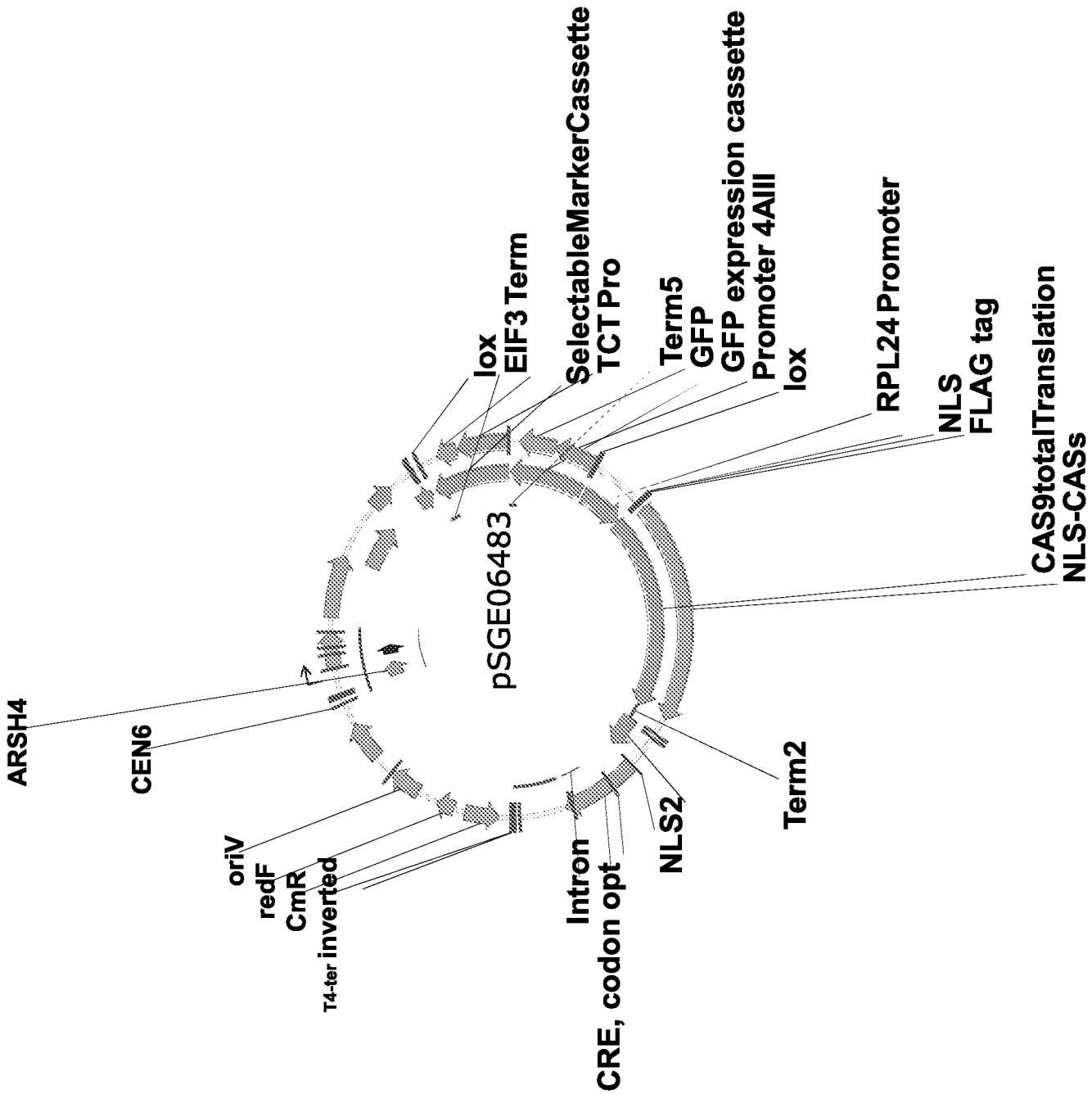

pSGE06483
ARSH4
CEN6
oriV
redF
CmR
T4-ter inverted
Intron
CRE, codon opt
NLS2
Term2
lox
EIF3 Term
SelectableMarkerCassette
TCT Pro
Term5
GFP
GFP expression cassette
Promoter 4AIII
lox
RPL24 Promoter
NLS
FLAG tag
CAS9totalTranslation
NLS-CASs

FIG. 18

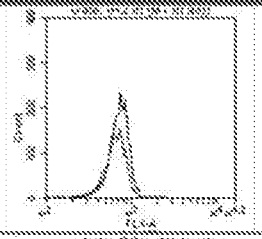
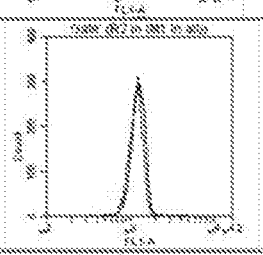
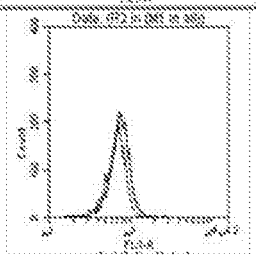
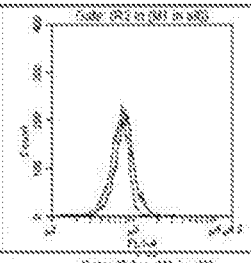
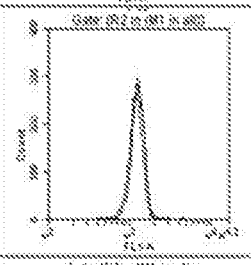
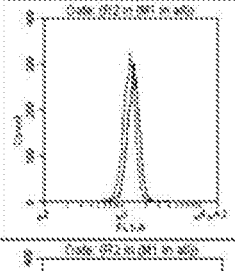
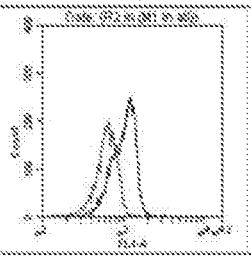
Sample		Mean Fluorescence (GFP)	
		NH_4	NO_3
WE-SGI-E-03730		728	661
pSGE06483-A11		1262	1180
pSGE06483-B11		773	683
pSGE06483-C12		807	667
pSGE06483-D12		1266	1195
pSGE06483-E12		1415	1248
pSGE06483-F12		1042	637

FIG. 19

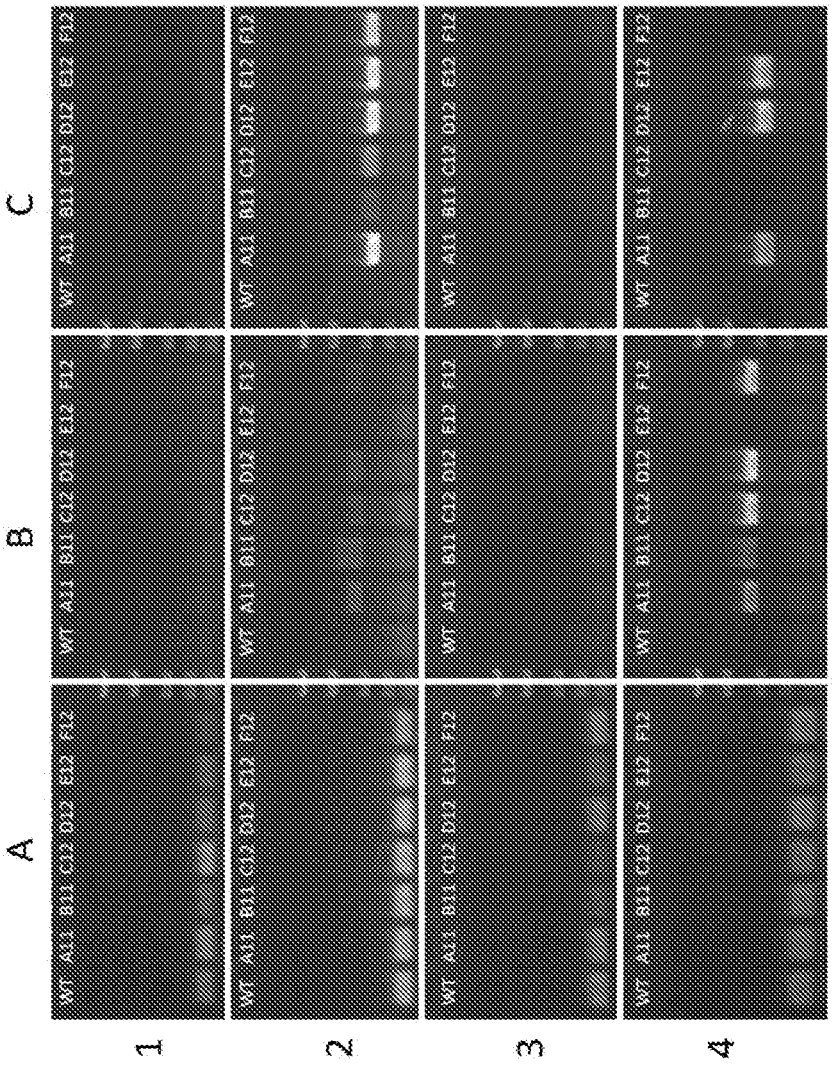


FIG. 20

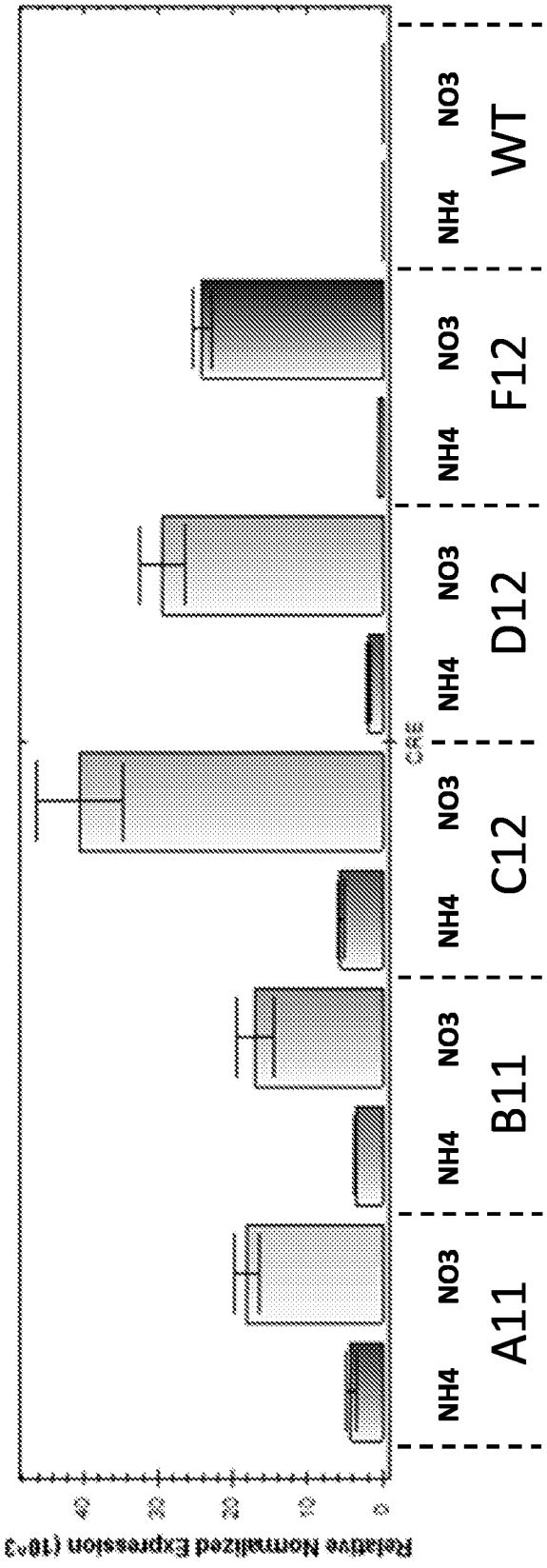

Relative Normalized Expression (10^3)
0
5
10
15
20
NH4
NO3
A11
B11
C12
D12
F12
WT
CSS

FIG. 21

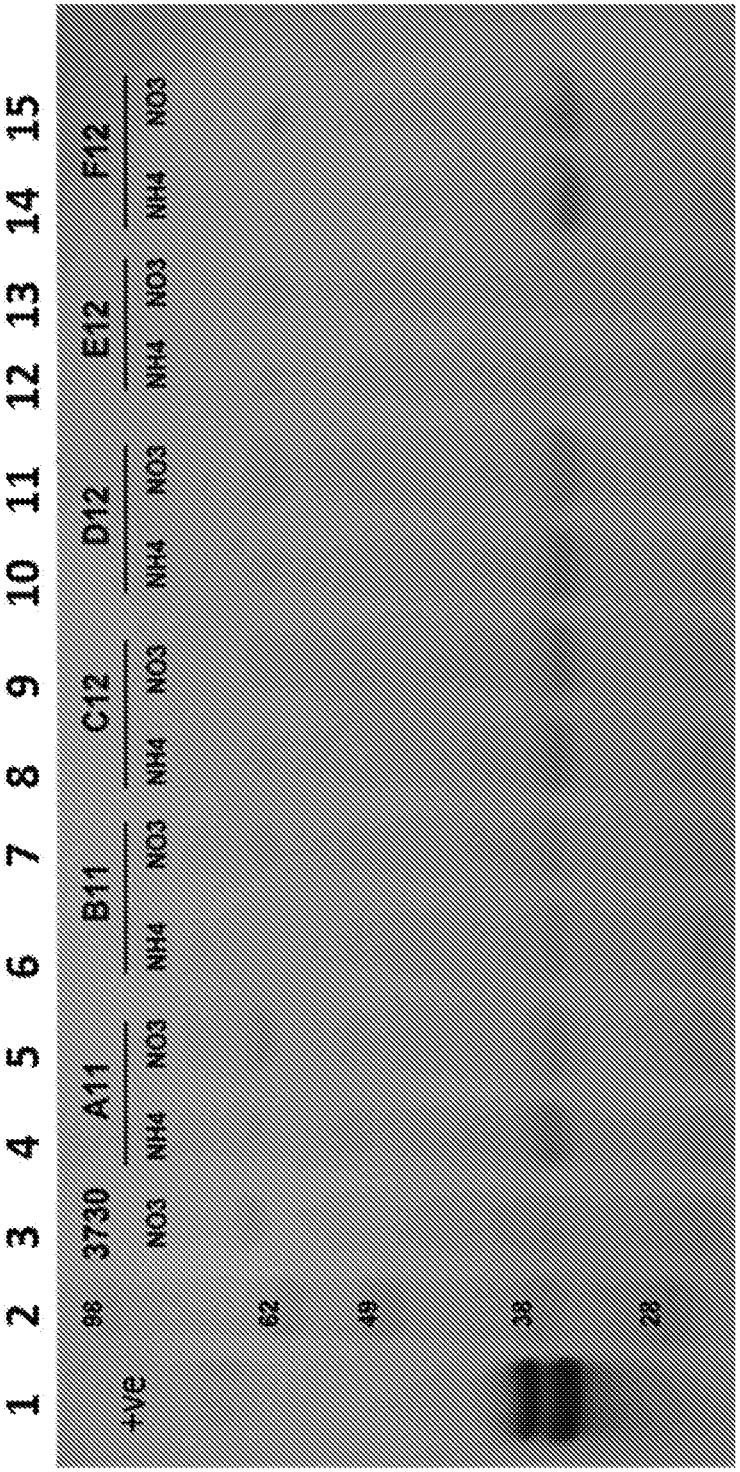

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
+ve
88
3730
NO3
NH4
A11
NO3
NH4
B11
NO3
NH4
C12
NO3
NH4
D12
NO3
NH4
E12
NO3
NH4
F12
NO3
NH4
62
49
38
28

FIG. 22

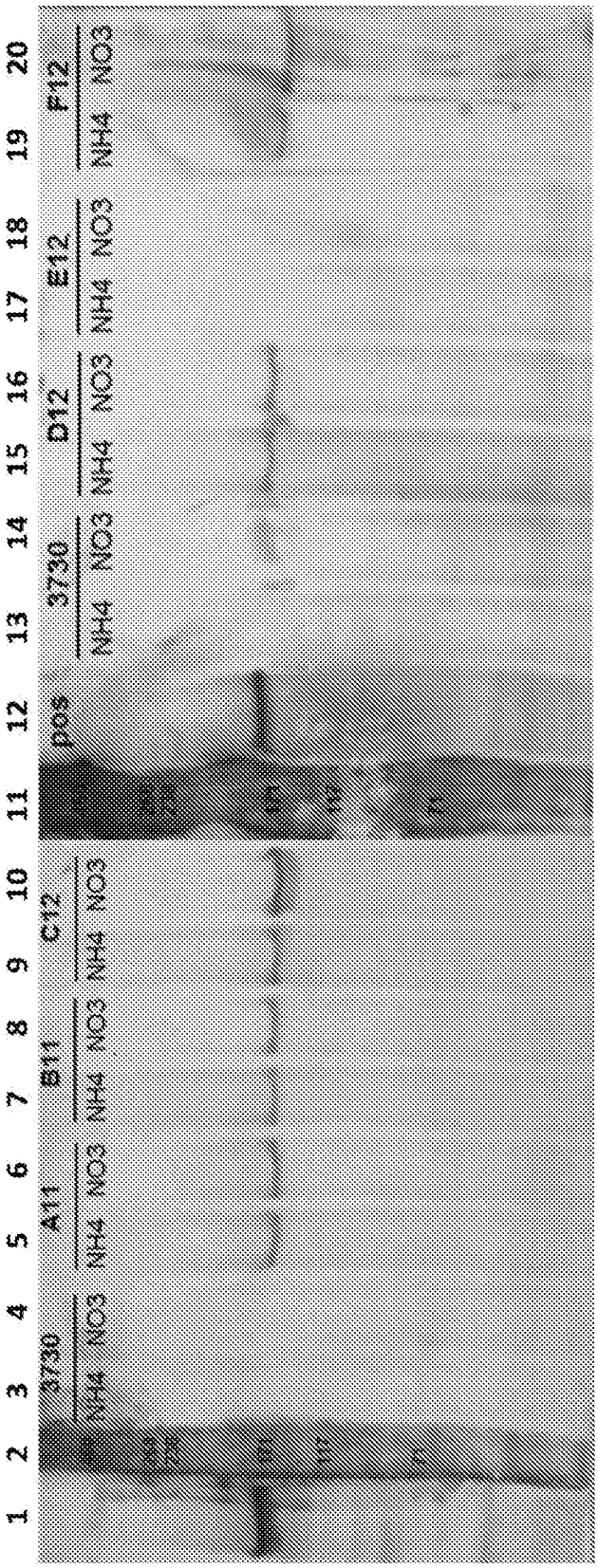

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
3730
NH4
NO3
A11
NH4
NO3
B11
NH4
NO3
C12
NH4
NO3
pos
3730
NH4
NO3
D12
NH4
NO3
E12
NH4
NO3
F12
NH4
NO3

FIG. 23

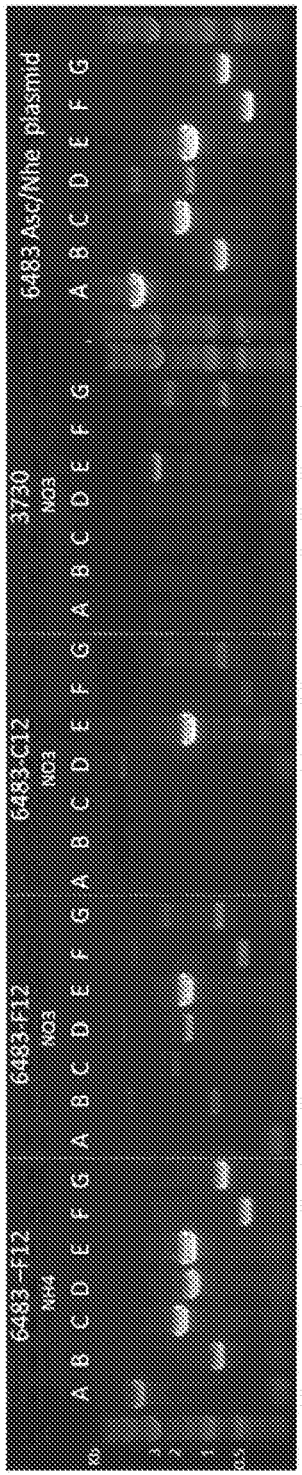

6493-F12
Xho1
6483-F12
Xho1
6483-G12
Xho1
3730
Xho3
6483 Asc/Nhe plasmid

FIG. 24

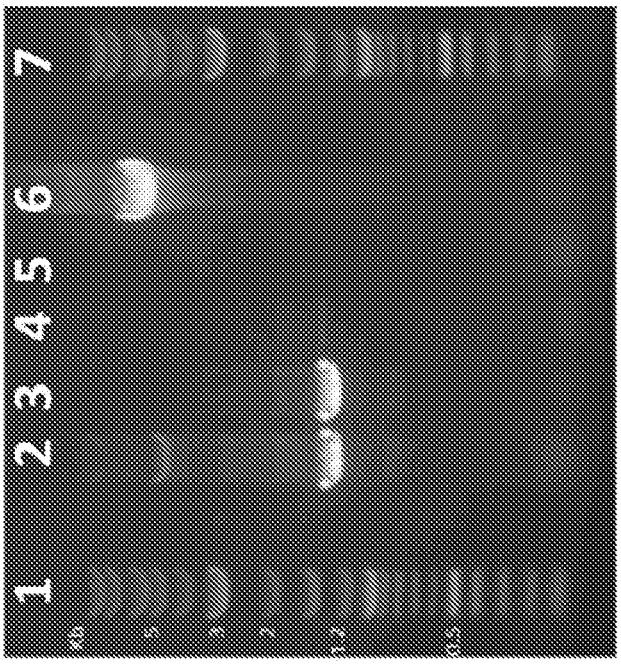


FIG. 25

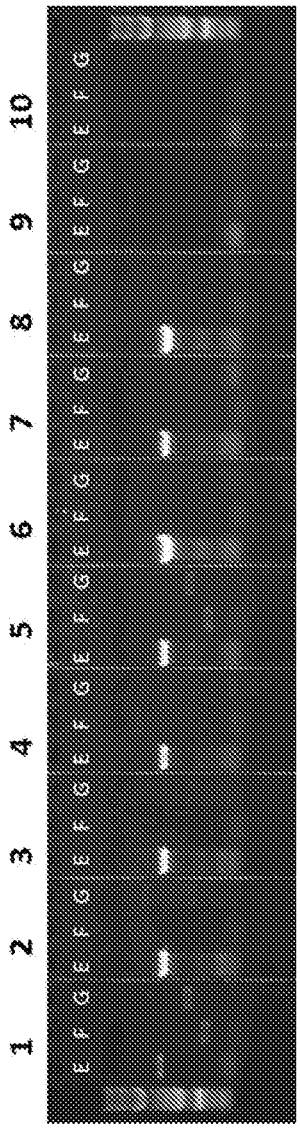
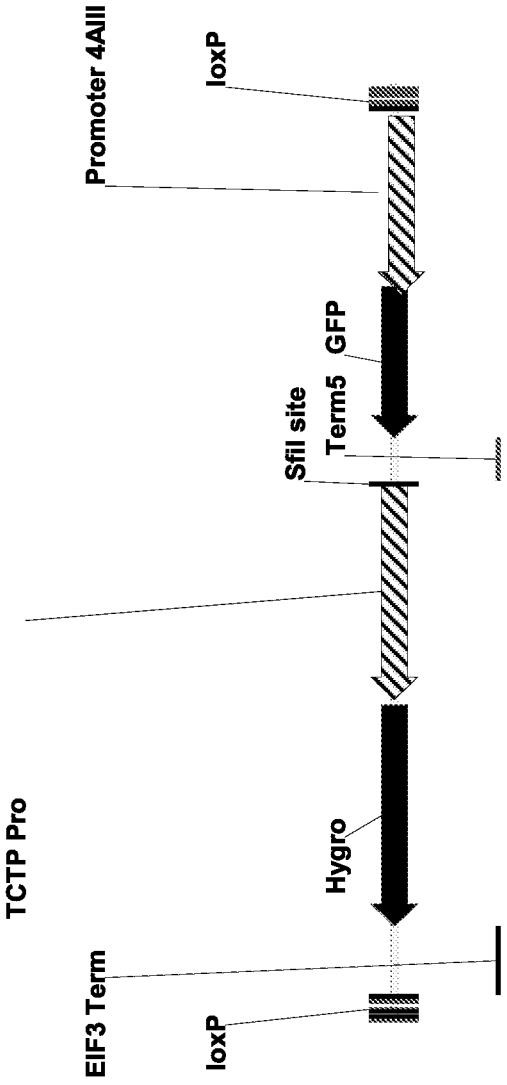


FIG. 26



loxP-HygR-GFP-loxP Cassette

FIG. 27

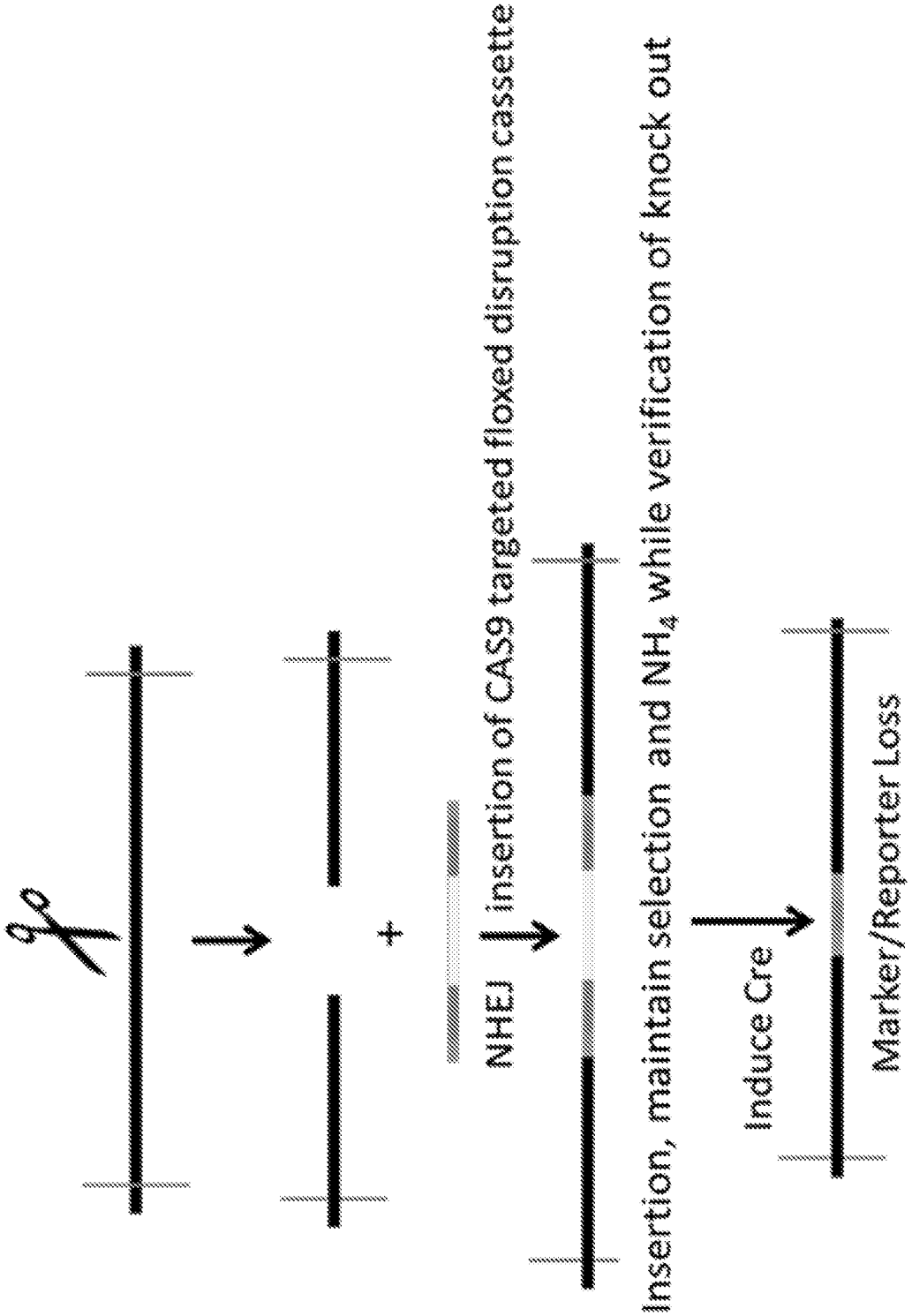

+
NHEJ
insertion of CAS9 targeted floxed disruption cassette
Insertion, maintain selection and NH4 while verification of knock out
Induce Cre
Marker/Reporter Loss

FIG. 28

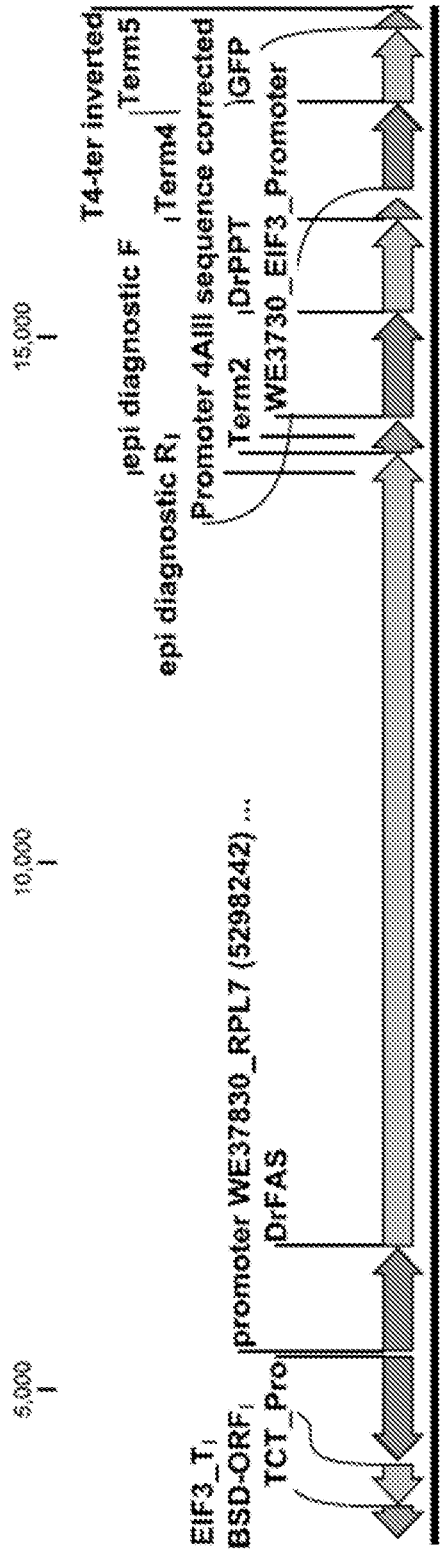

5,000
10,000
15,000
EIF3_T1
BSD-ORF1
TCT_Pro
DrFAS
promoter WE37830_RPL7 (5298242) ...
epi diagnostic R1
epi diagnostic F
Promoter 4AIII sequence corrected
Term2
DrPPT
WE3730_EIF3_Promoter
GFP
Term4
T4-ter inverted
Term5

FIG. 29

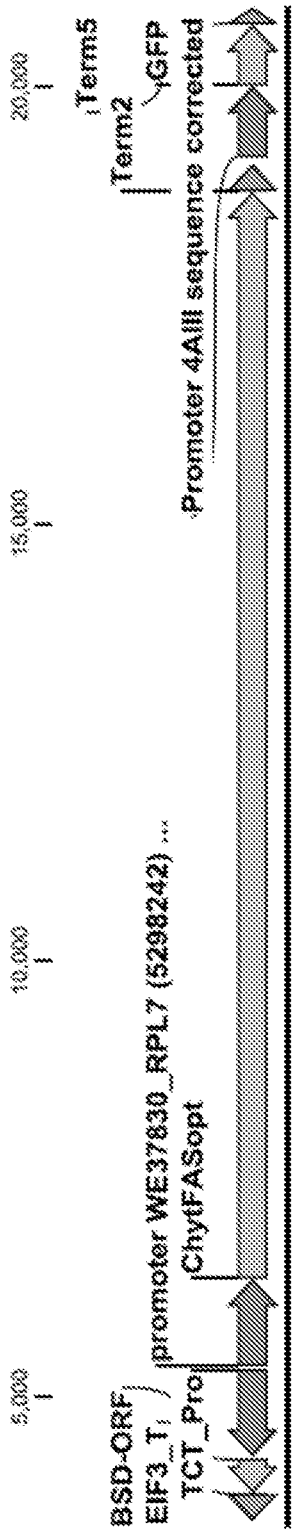

5,000
10,000
15,000
20,000
BSD-ORF
EIF3_T1
TCT_Pro
promoter WE37830_RPL7 (5298242) ...
ChytFASopt
Promoter 4AIII sequence corrected
Term2
GFP
Term5

FIG. 30

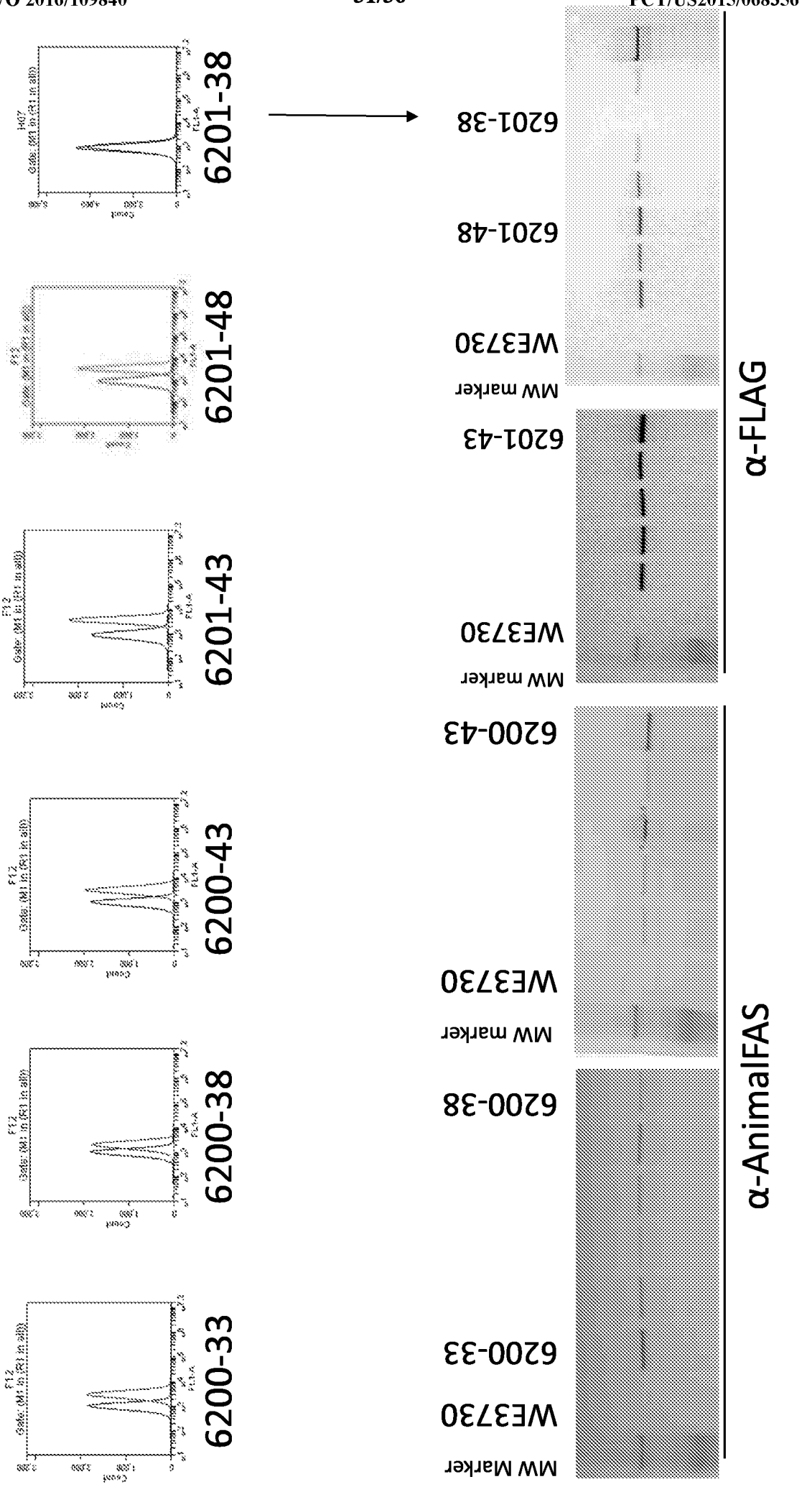

6200-33
6200-38
6200-43
6201-43
6201-48
6201-38
MW Marker
WE3730
6200-33
6200-38
MW marker
WE3730
6200-43
MW marker
WE3730
6201-43
MW marker
WE3730
6201-48
6201-38
α-AnimalFAS
α-FLAG

FIG. 31

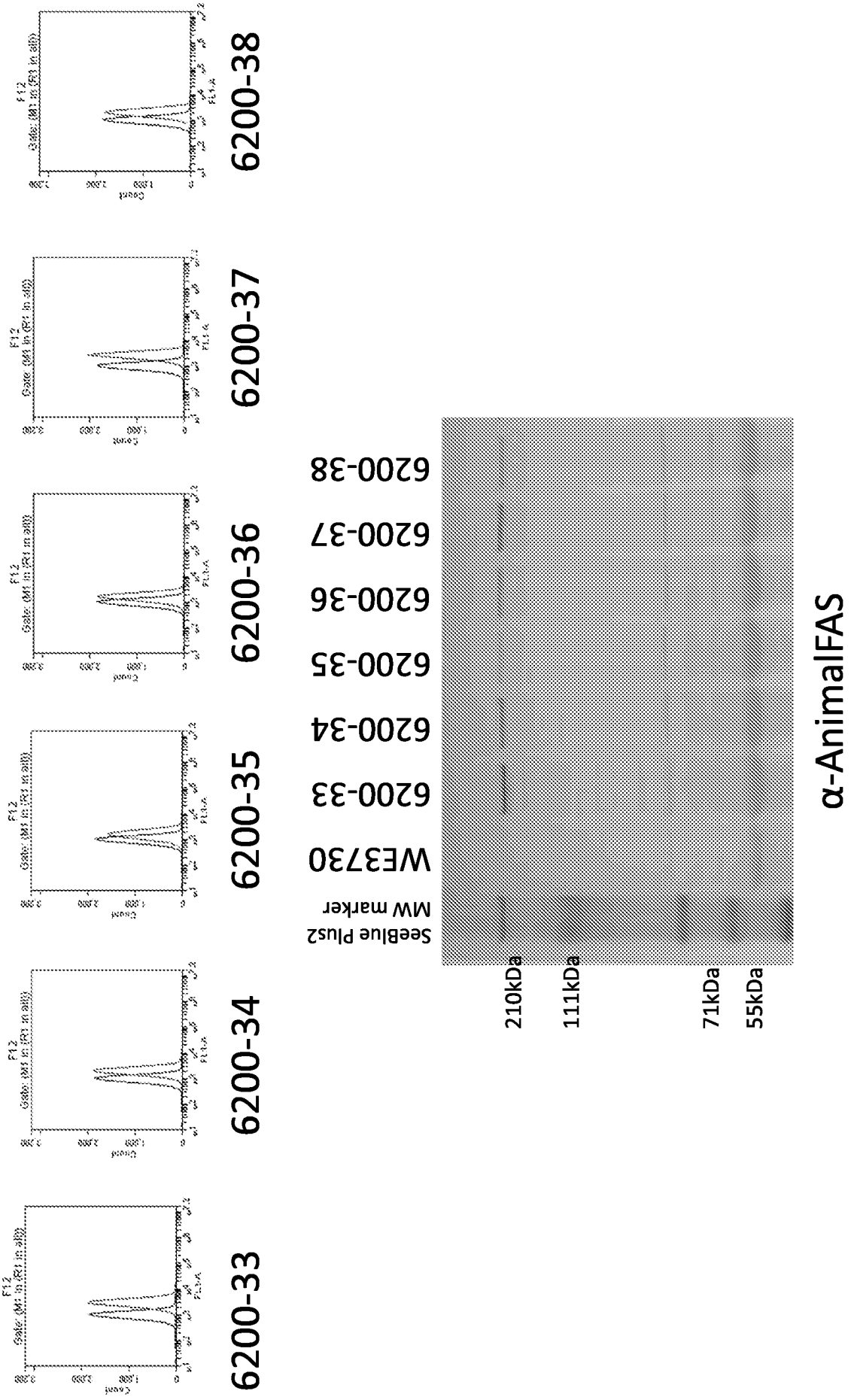

6200-33
6200-34
6200-35
6200-36
6200-37
6200-38
WE3730
6200-33
6200-34
6200-35
6200-36
6200-37
6200-38
SeeBlue Plus2
MW marker
210kDa
111kDa
71kDa
55kDa
α-AnimalFAS

FIG. 32

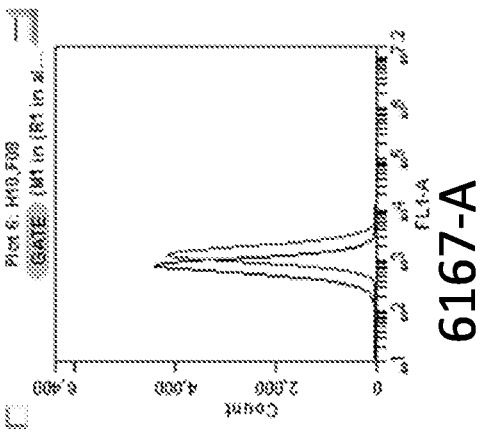

6167-A
6167-B
HiMark MW marker
W/E3730
6167-A
6167-B
Chytrid # 5554
460kDa
268kDa
238kDa
α-ChytridFAS

FIG. 33

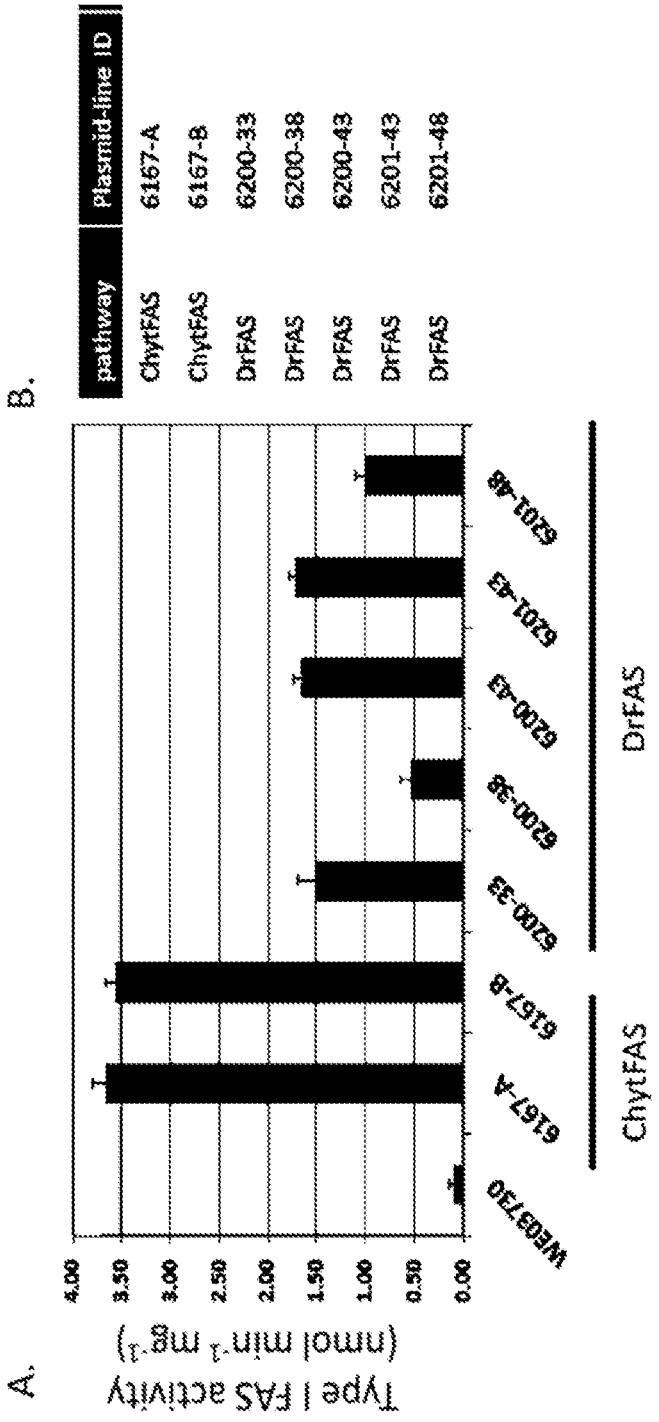

A.
Type I FAS activity
(nmol min-1 mg-1)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
WE0573O
6167-A
6167-8
6200-33
6200-38
6200-43
6201-43
6201-48
ChytFAS
DrFAS
B.
pathway
Plasmid-line ID
ChytFAS 6167-A
ChytFAS 6167-8
DrFAS 6200-33
DrFAS 6200-38
DrFAS 6200-43
DrFAS 6201-43
DrFAS 6201-48

FIG. 34

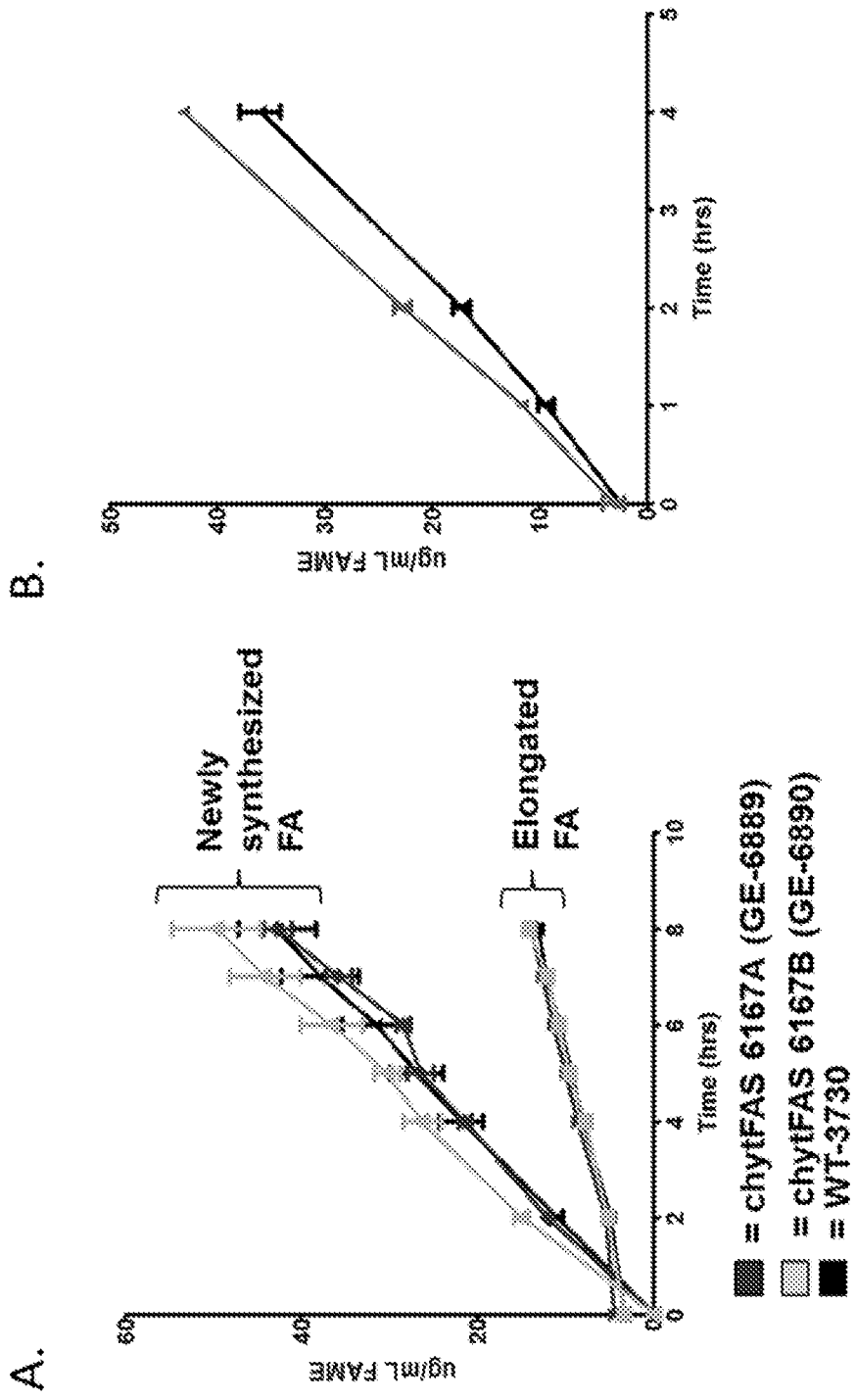
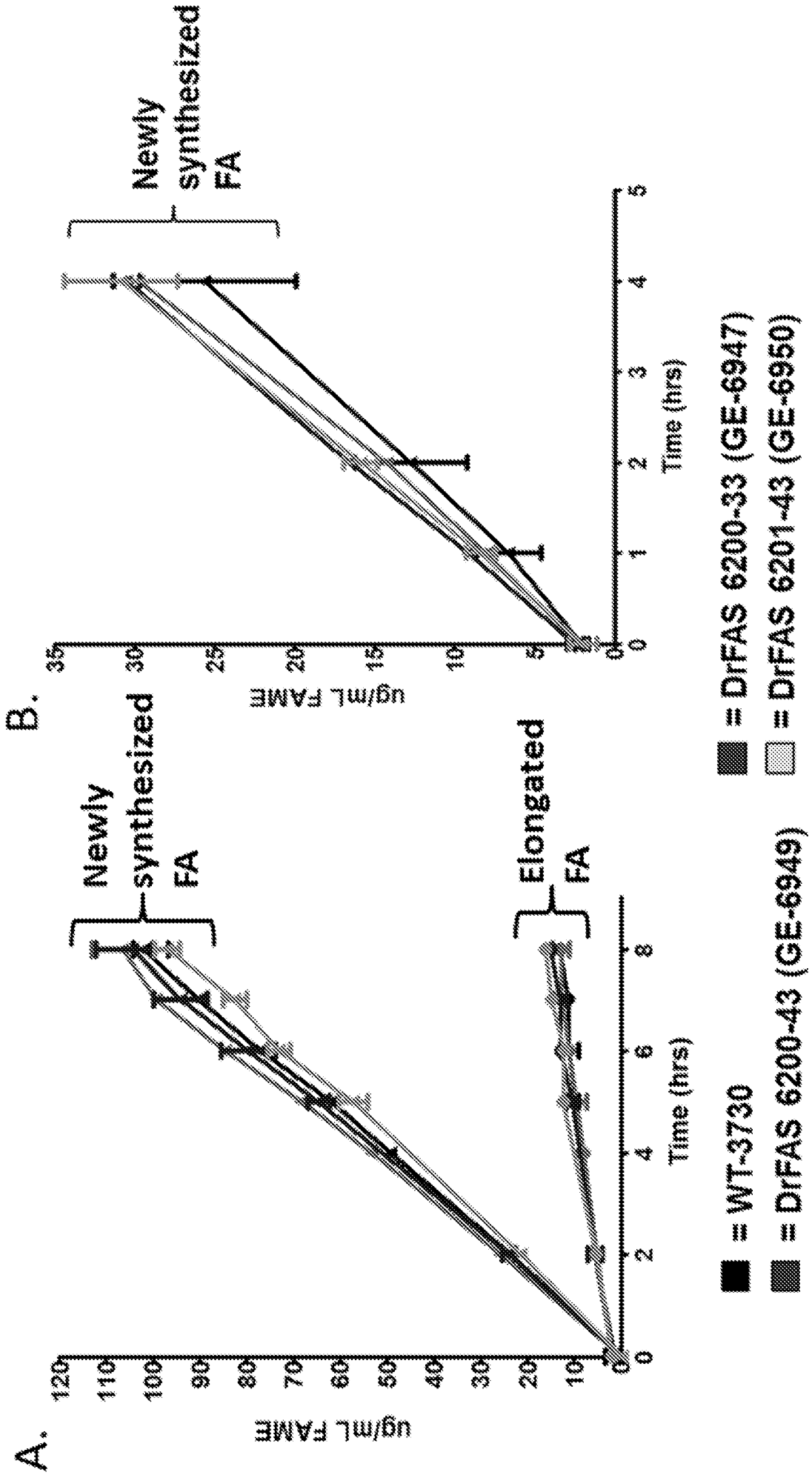

A.
B.
ug/mL FAME
Time (hrs)
Newly synthesized FA
Elongated FA
= chytFAS 6167A (GE-6889)
= chytFAS 6167B (GE-6890)
= WT-3730

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