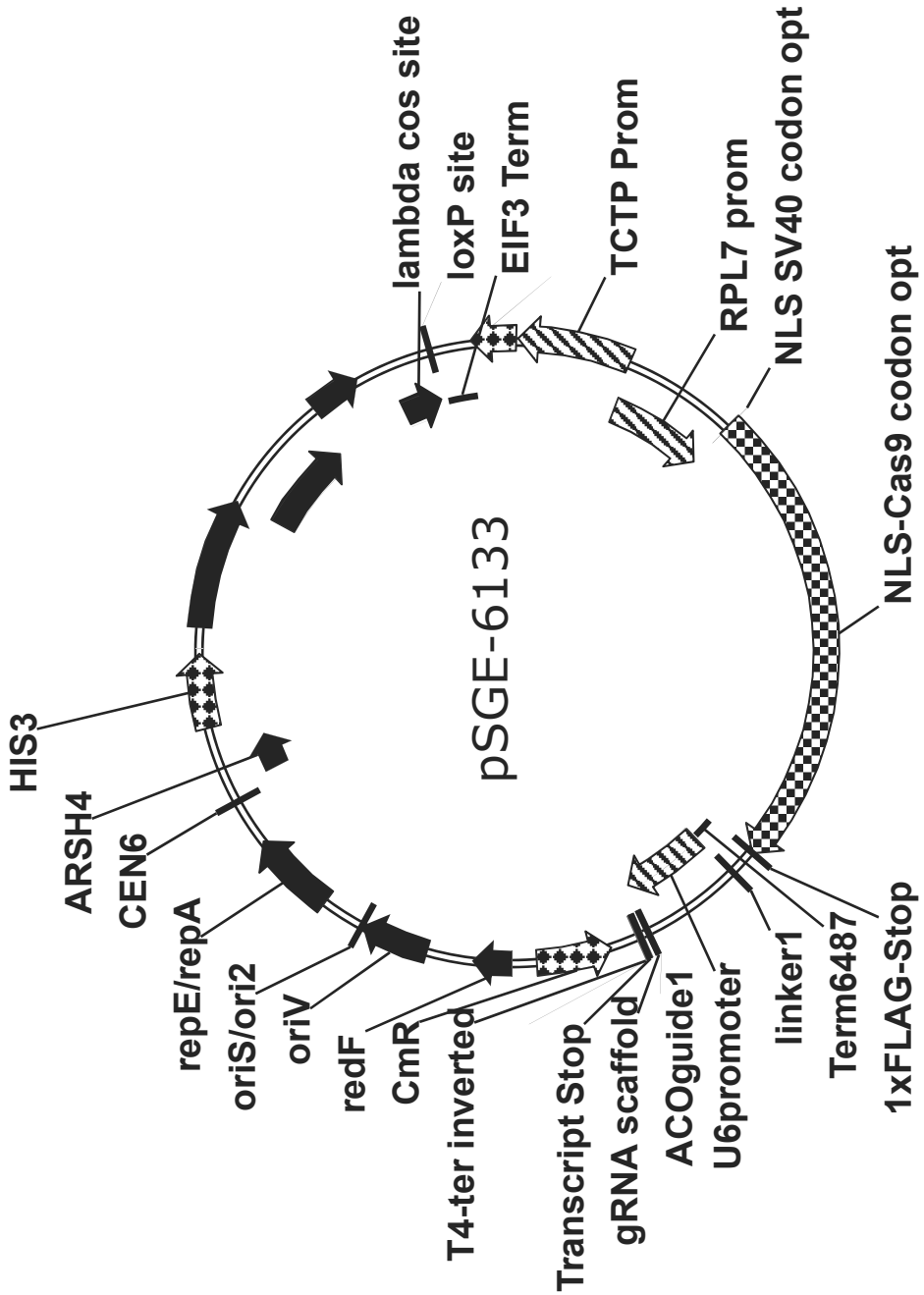

pSGE-6133
lambda cos site
loxP site
EIF3 Term
TCTP Prom
RPL7 prom
NLS SV40 codon opt
NLS-Cas9 codon opt
1xFLAG-Stop
Term6487
linker1
U6promoter
ACOguide1
gRNA scaffold
Transcript Stop
T4-ter inverted
CmR
redF
oriV
oriS/ori2
repE/repA
CEN6
ARSH4
HIS3

FIG. 1

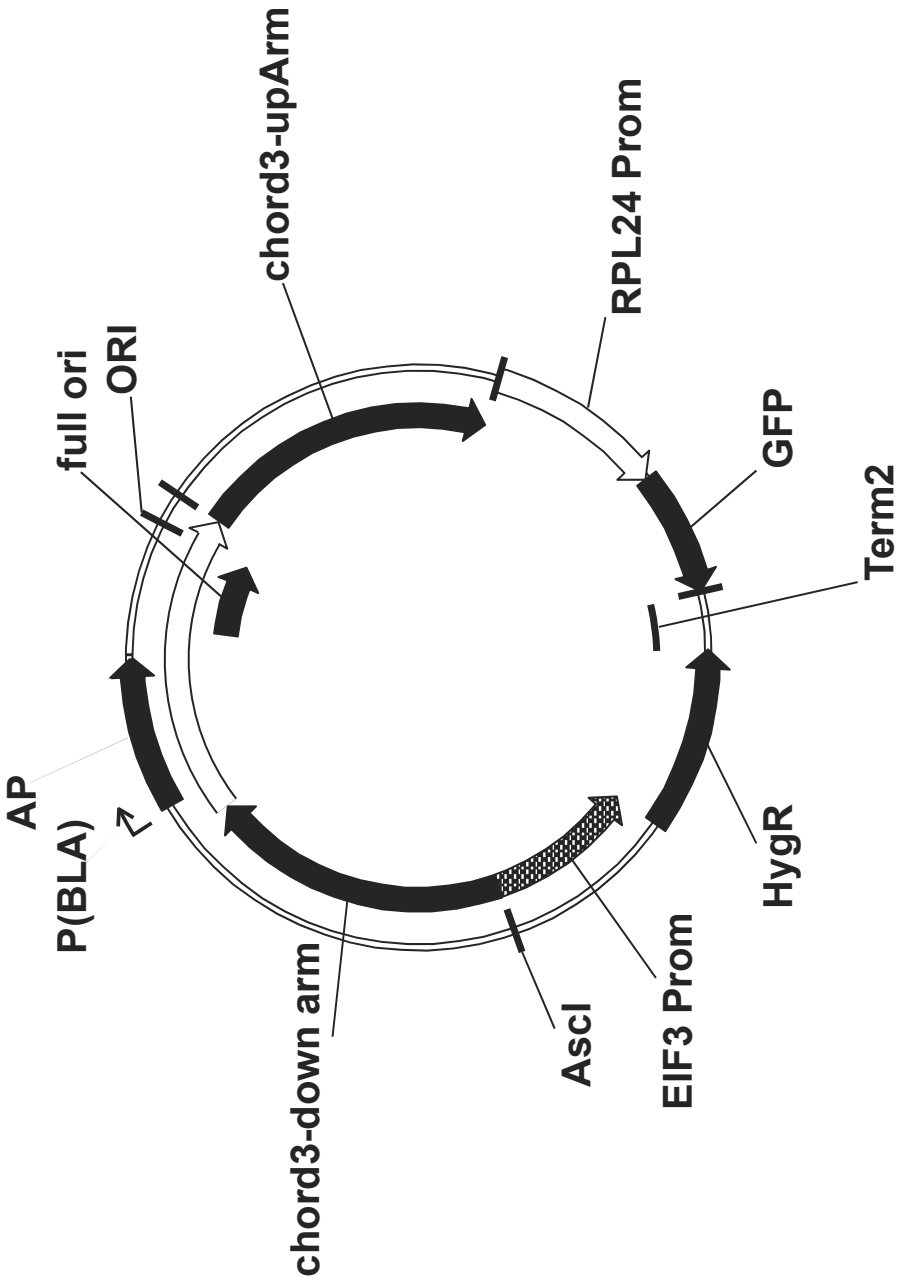

chord3-upArm
RPL24 Prom
GFP
Term2
HygR
EIF3 Prom
AscI
chord3-down arm
P(BLA)
AP
full ori
ORI

FIG. 2

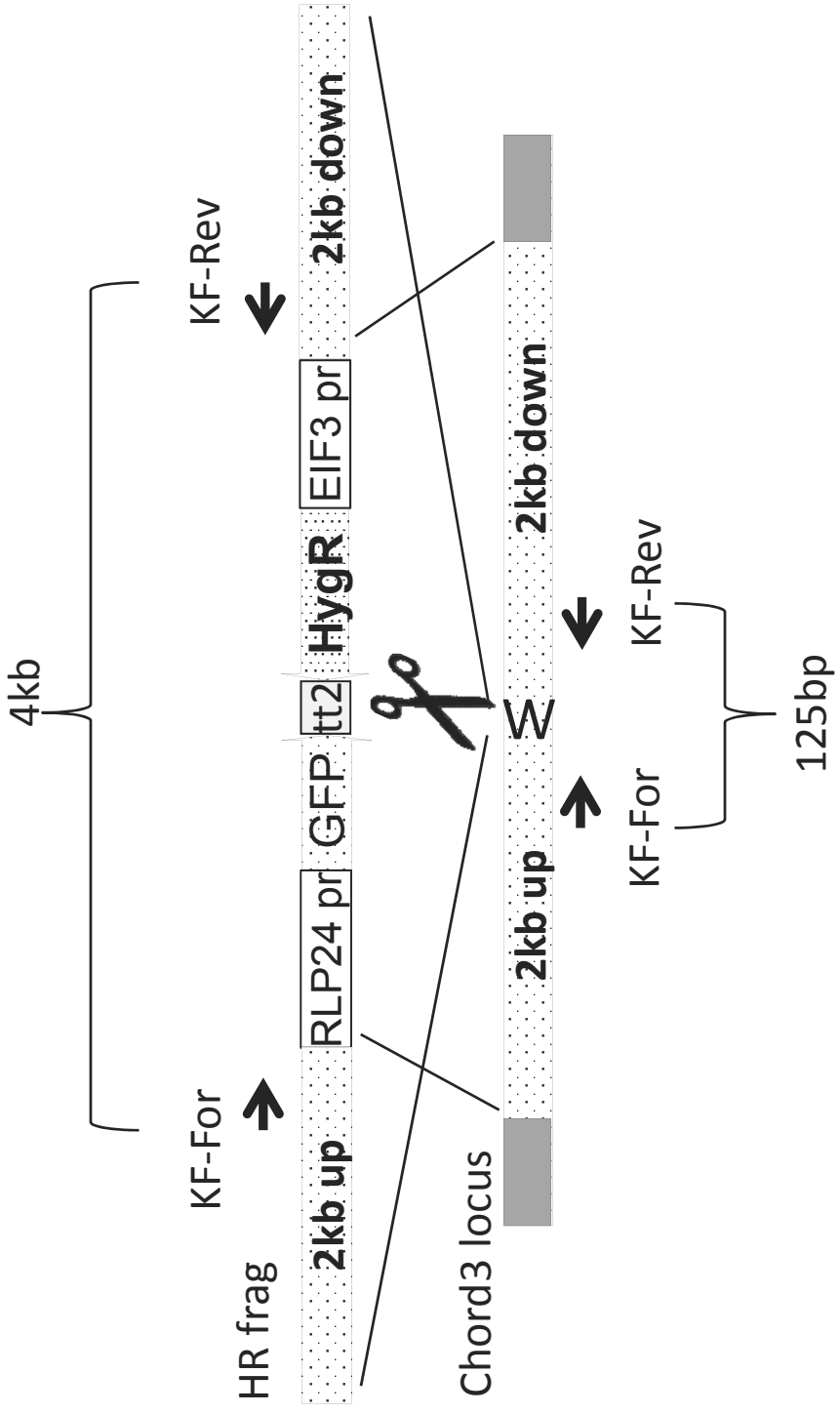

4kb
KF-Rev
KF-For
HR frag
2kb up
RLP24 pr
GFP
tt2
HygR
EIF3 pr
2kb down
Chord3 locus
2kb up
2kb down
KF-Rev
KF-For
125bp

FIG. 3

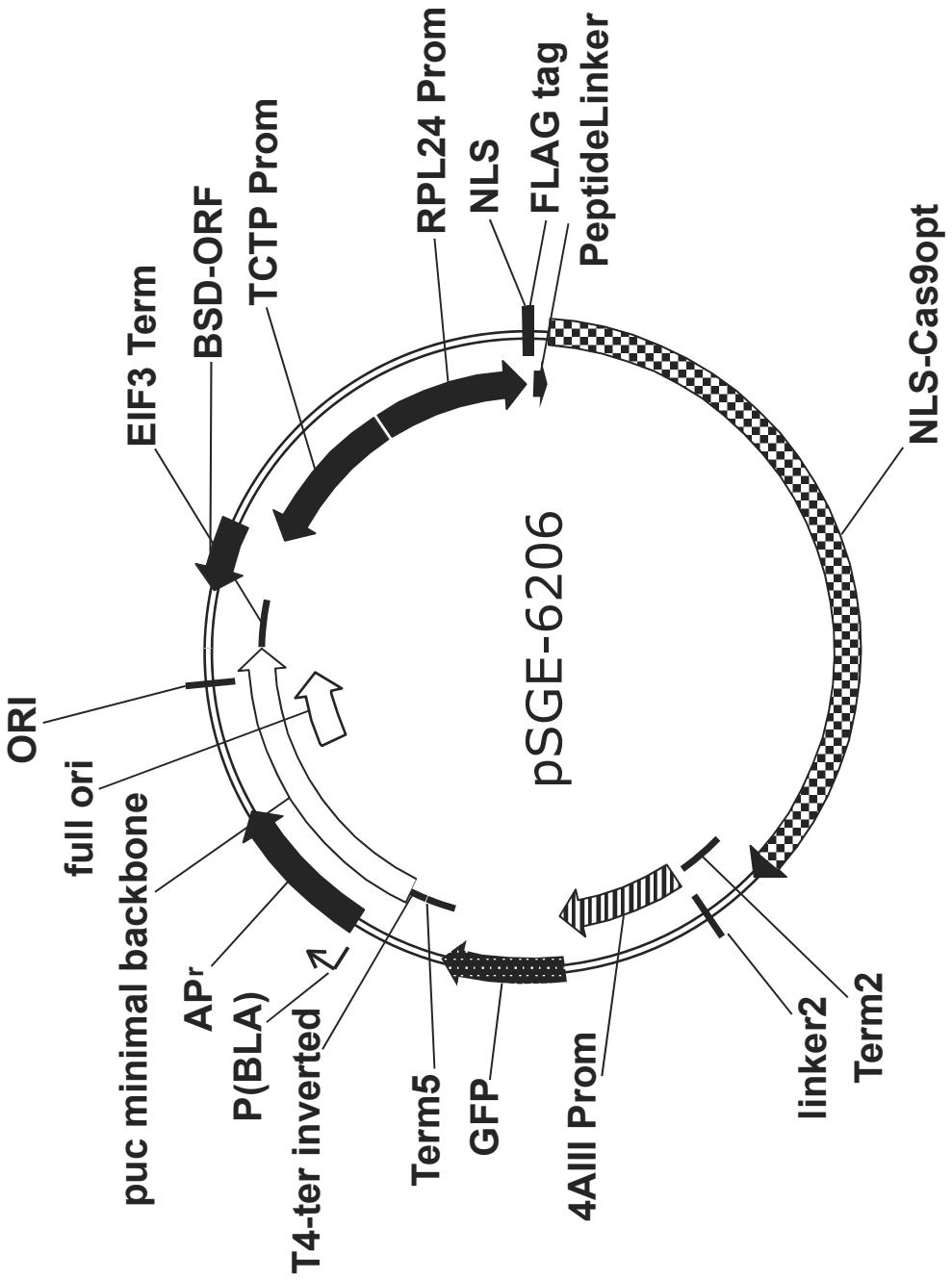
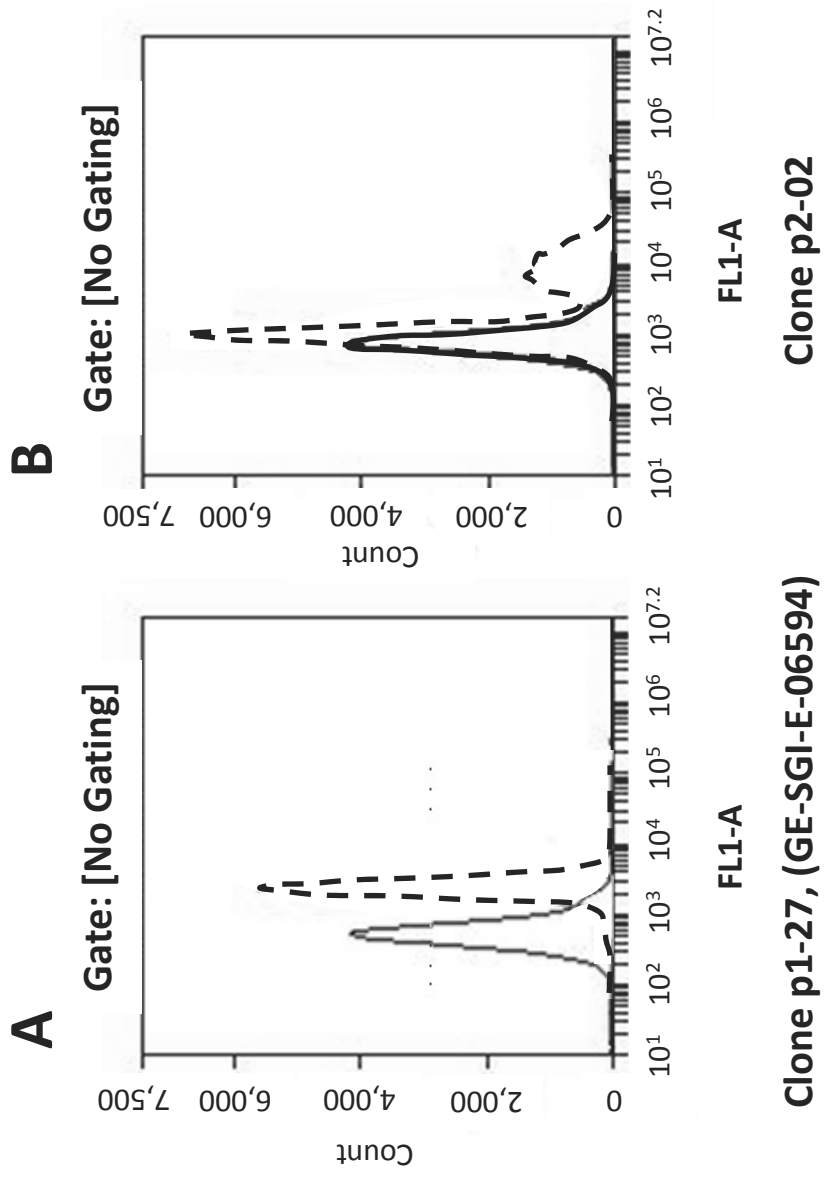

pSGE-6206
ORI
full ori
puc minimal backbone
APr
P(BLA)
T4-ter inverted
Term5
GFP
4AIII Prom
linker2
Term2
NLS-Cas9opt
EIF3 Term
BSD-ORF
TCTP Prom
RPL24 Prom
NLS
FLAG tag
PeptideLinker

FIG. 4


A
Gate: [No Gating]
Count
0
2,000
4,000
6,000
7,500
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
FL1-A
Clone p1-27, (GE-SGI-E-06594)
B
Gate: [No Gating]
Count
0
2,000
4,000
6,000
7,500
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
FL1-A
Clone p2-02

FIGS. 5A-B

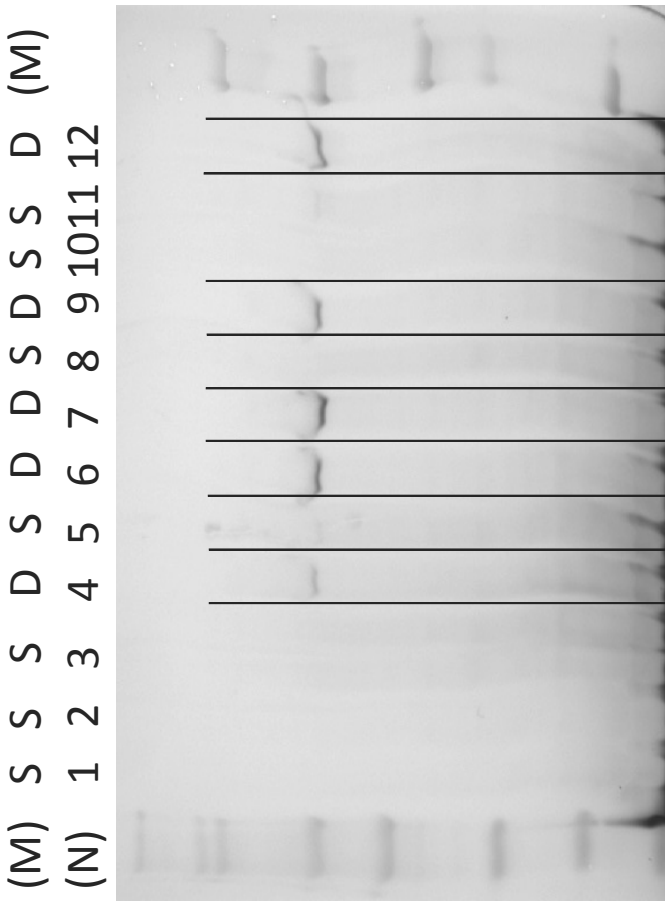


FIG. 6

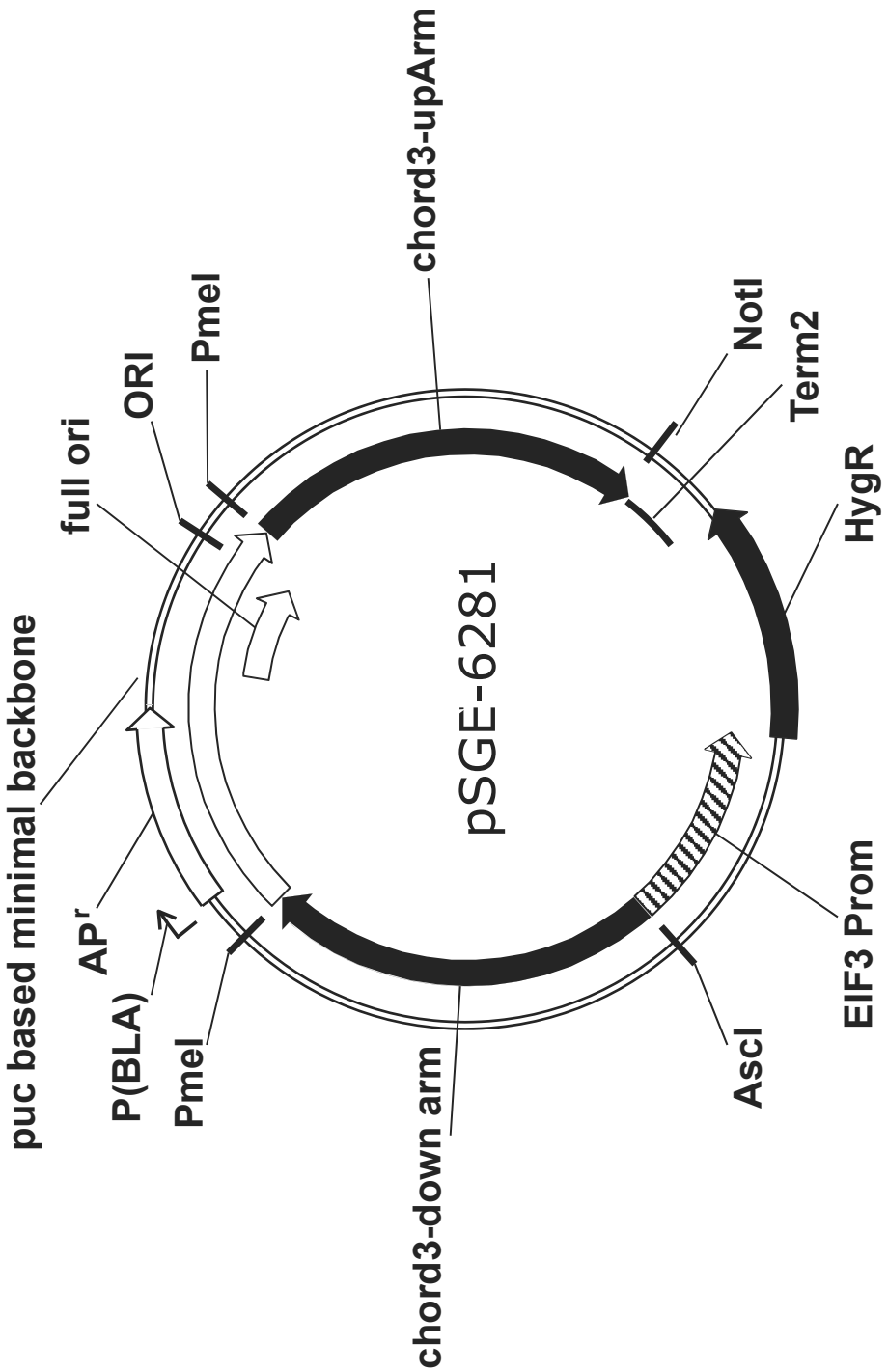

pSGE-6281
chord3-upArm
NotI
Term2
HygR
EIF3 Prom
AscI
chord3-down arm
PmeI
P(BLA)
AP^r
puc based minimal backbone
full ori
ORI
PmeI

FIG. 7

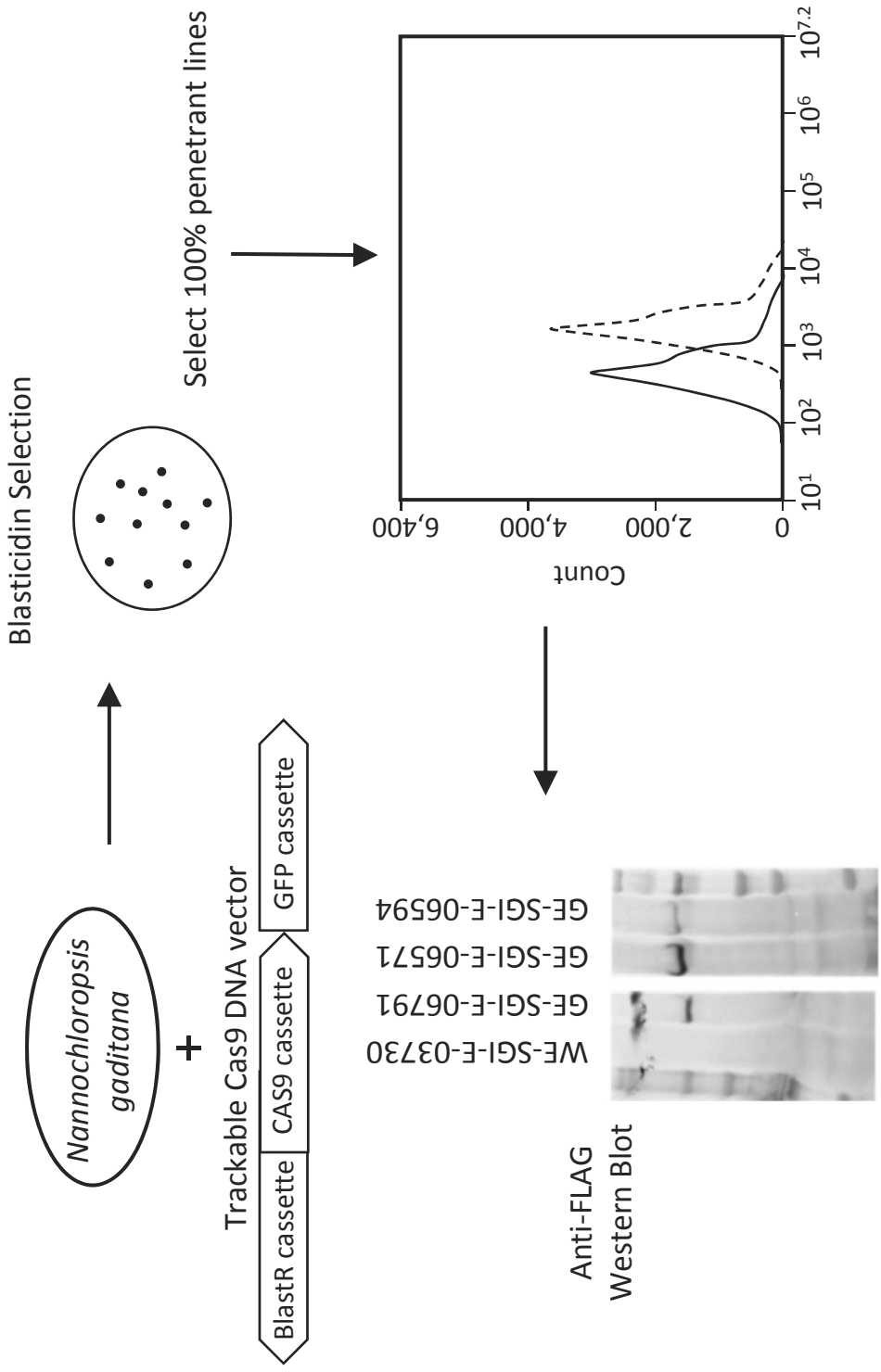

Nannochloropsis gaditana
+
Trackable Cas9 DNA vector
BlastR cassette
CAS9 cassette
GFP cassette
Blasticidin Selection
Select 100% penetrant lines
Count
0
2,000
4,000
6,400
10^1
10^2
10^3
10^4
10^5
10^6
$10^{7.2}$
Anti-FLAG
Western Blot
WE-SGI-E-03730
GE-SGI-E-06791
GE-SGI-E-06571
GE-SGI-E-06594

FIG. 8

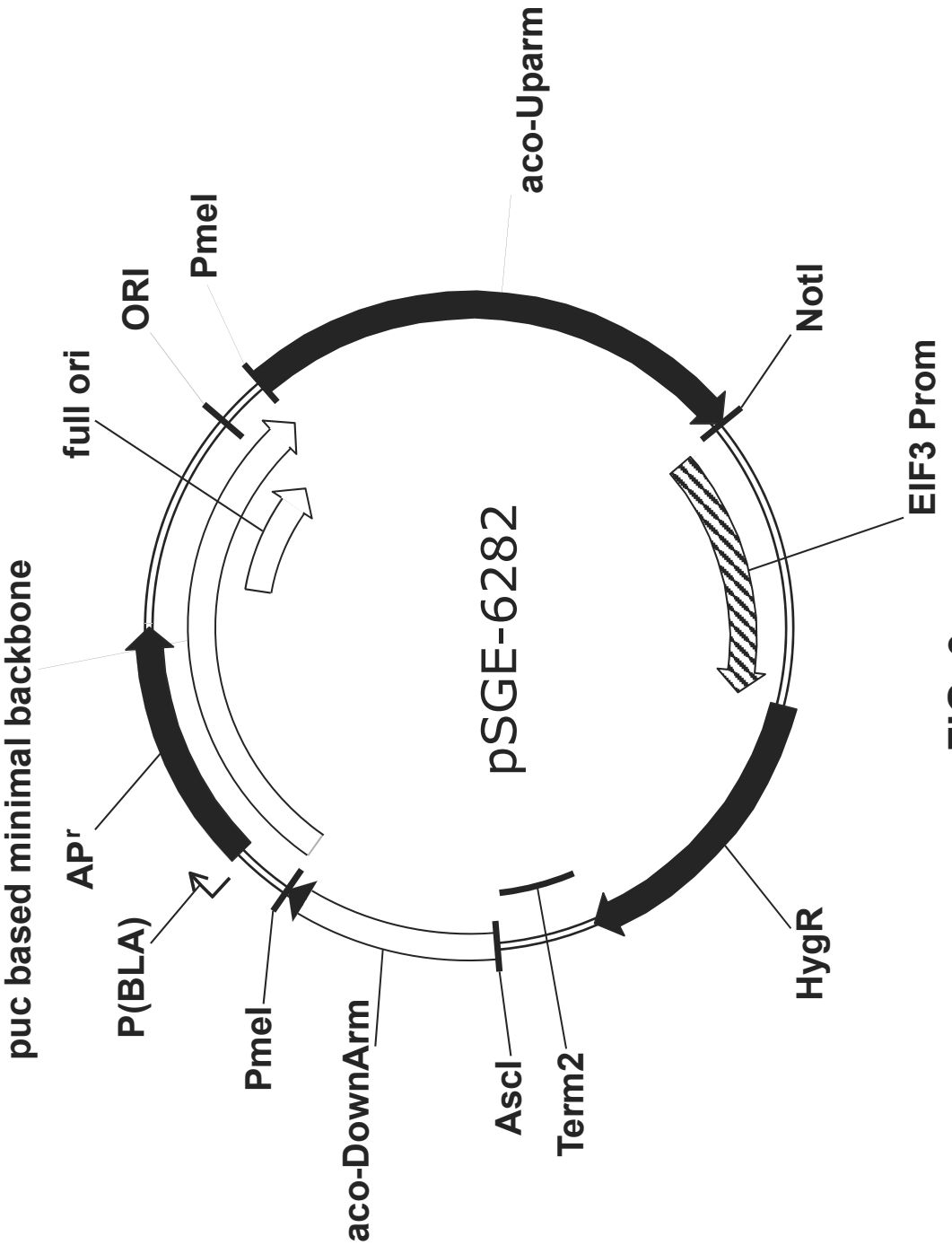

pSGE-6282
aco-Uparm
NotI
EIF3 Prom
HygR
Term2
AscI
aco-DownArm
PmeI
P(BLA)
AP^r
puc based minimal backbone
full ori
ORI
PmeI

FIG. 9

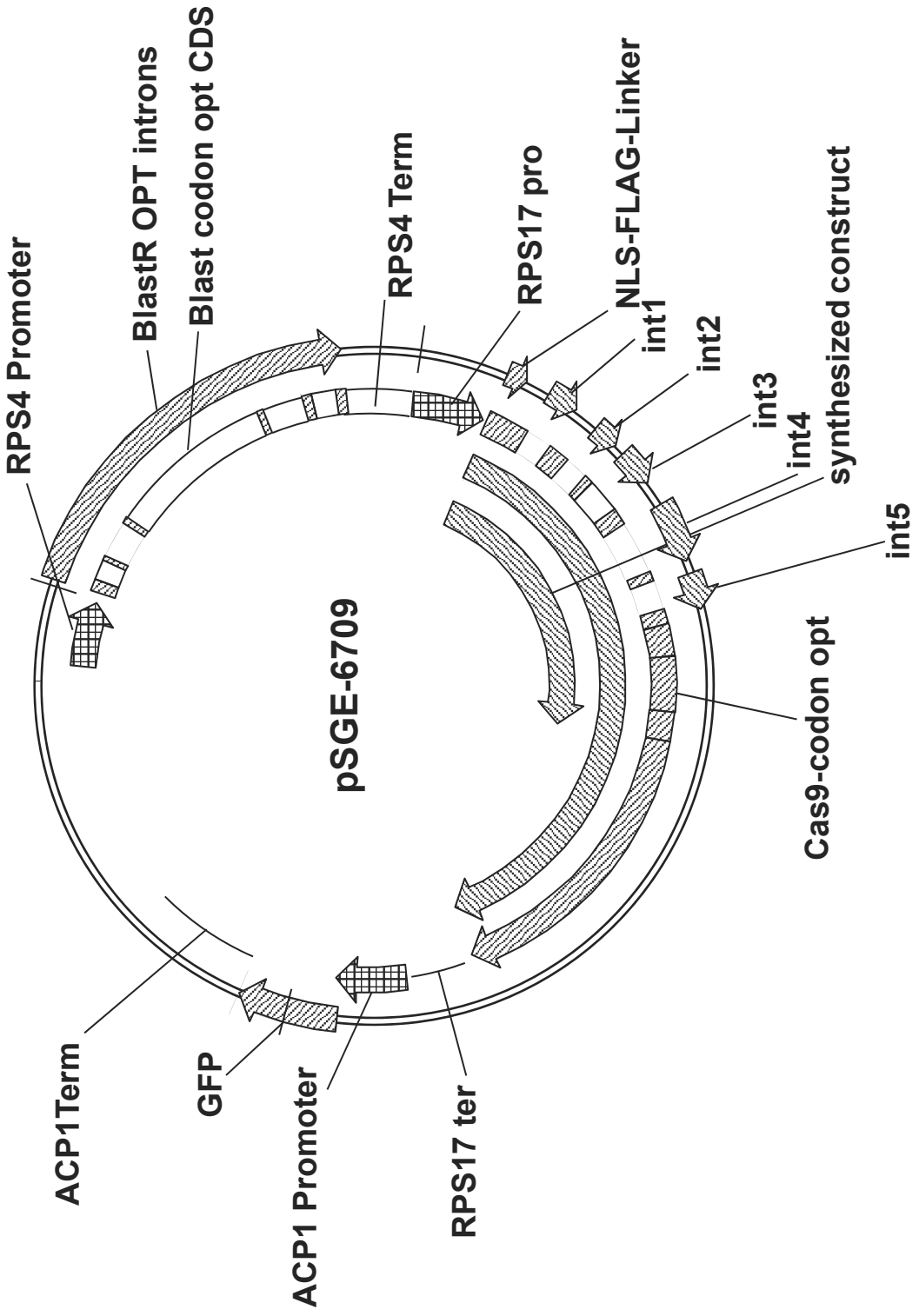

RPS4 Promoter
BlastR OPT introns
Blast codon opt CDS
RPS4 Term
RPS17 pro
NLS-FLAG-Linker
int1
int2
int3
int4
synthesized construct
int5
Cas9-codon opt
pSGE-6709
ACP1Term
GFP
ACP1 Promoter
RPS17 ter

FIG. 10

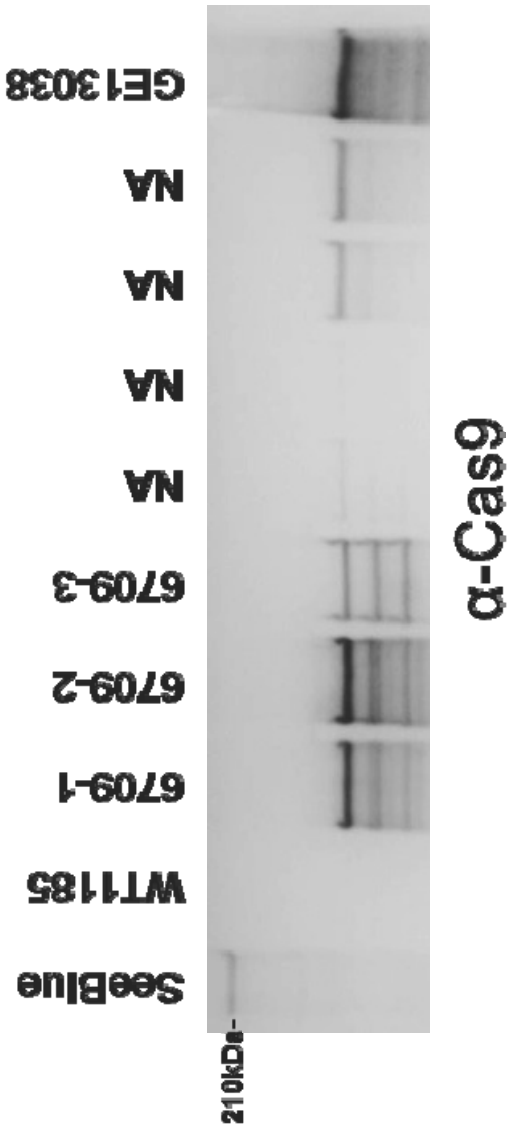

GE13038
NA
NA
NA
NA
6709-3
6709-2
6709-1
WT1185
SeeBlue
210kDa
α-Cas9

FIG. 11

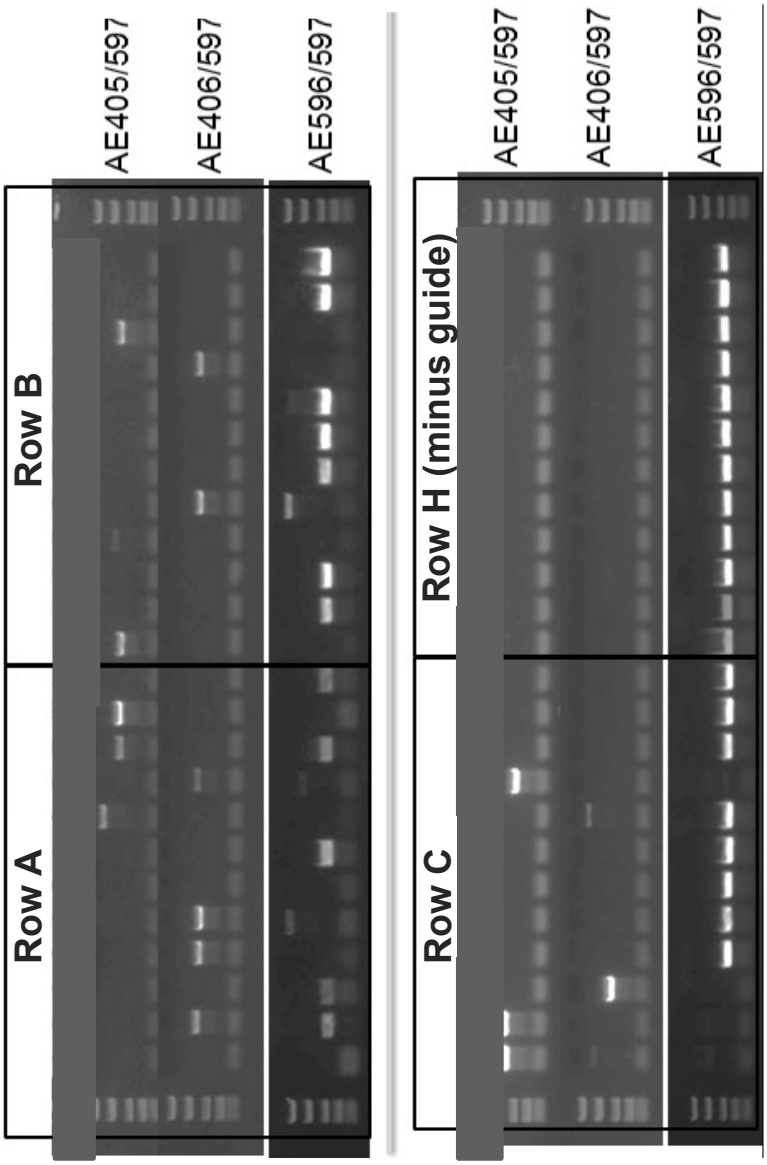
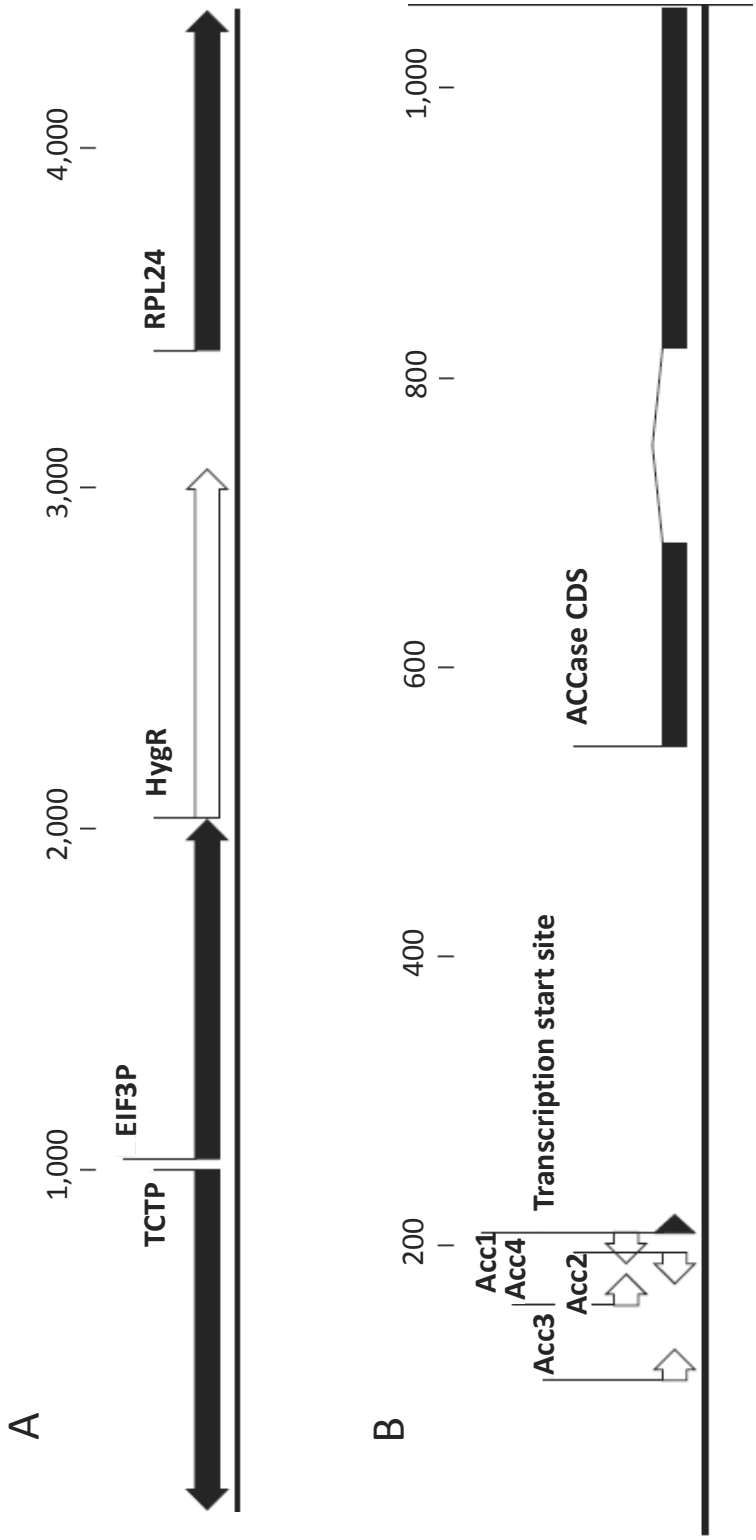
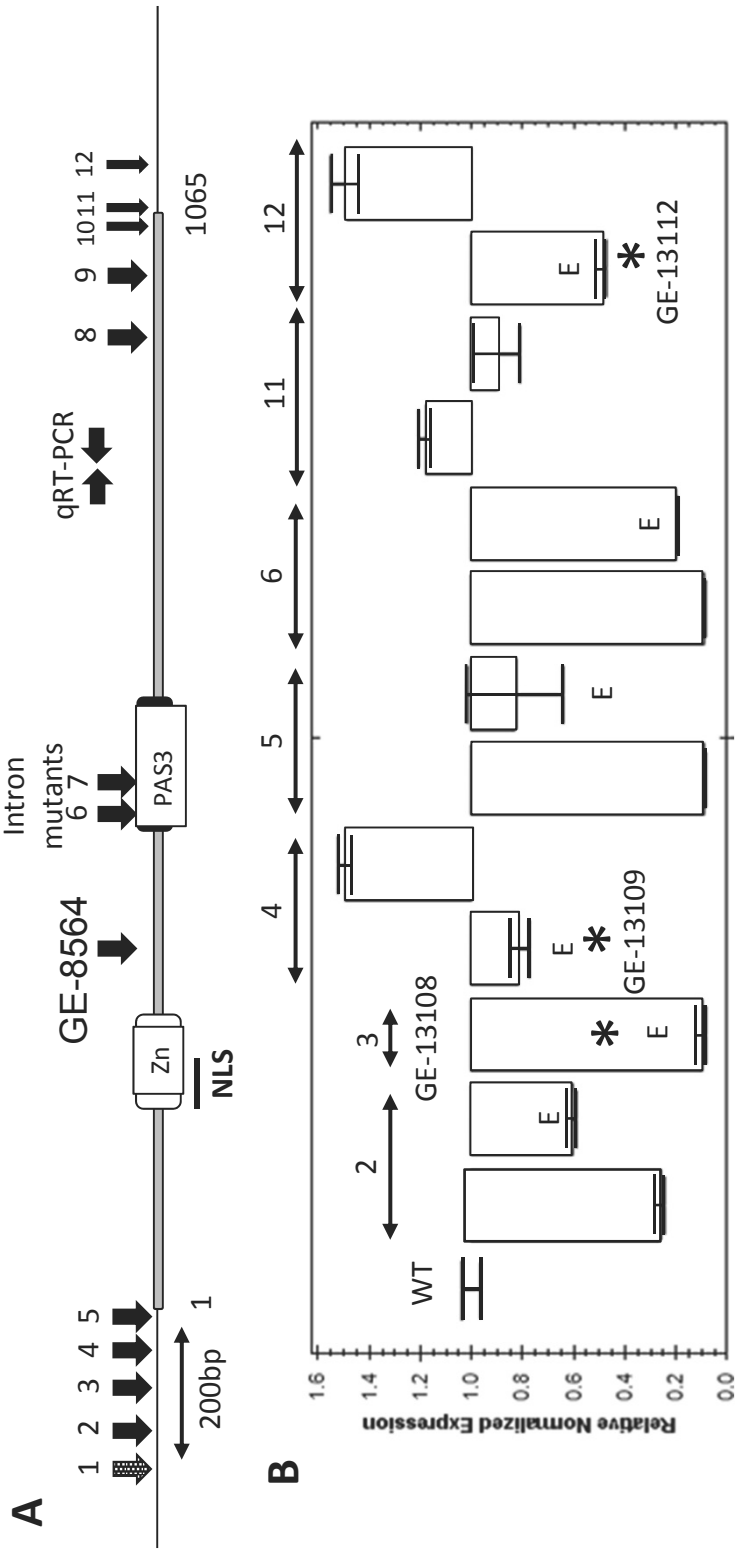

AE405/597
AE406/597
AE596/597
Row A
Row B
AE405/597
AE406/597
AE596/597
Row C
Row H (minus guide)

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-B


A
1 2 3 4 5
200bp
1
Zn
NLS
GE-8564
Intron mutants
6 7
PAS3
qRT-PCR
8 9 10 11 12
1065
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
GE-13109
5
6
11
12
GE-13112
E
*

FIGS. 14A-B

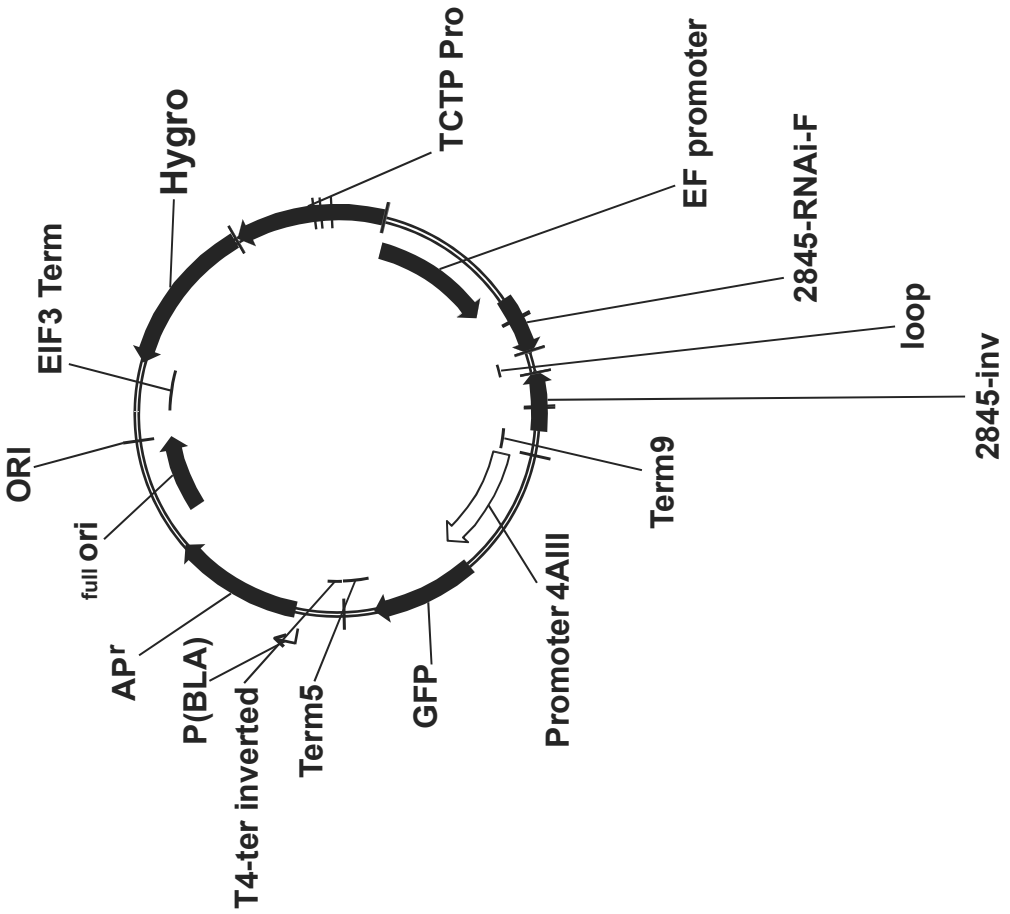

TCTP Pro
EF promoter
2845-RNAi-F
loop
2845-inv
Term9
Promoter 4AIII
GFP
Term5
T4-ter inverted
P(BLA)
AP^r
full ori
ORI
EIF3 Term
Hygro

FIG. 15

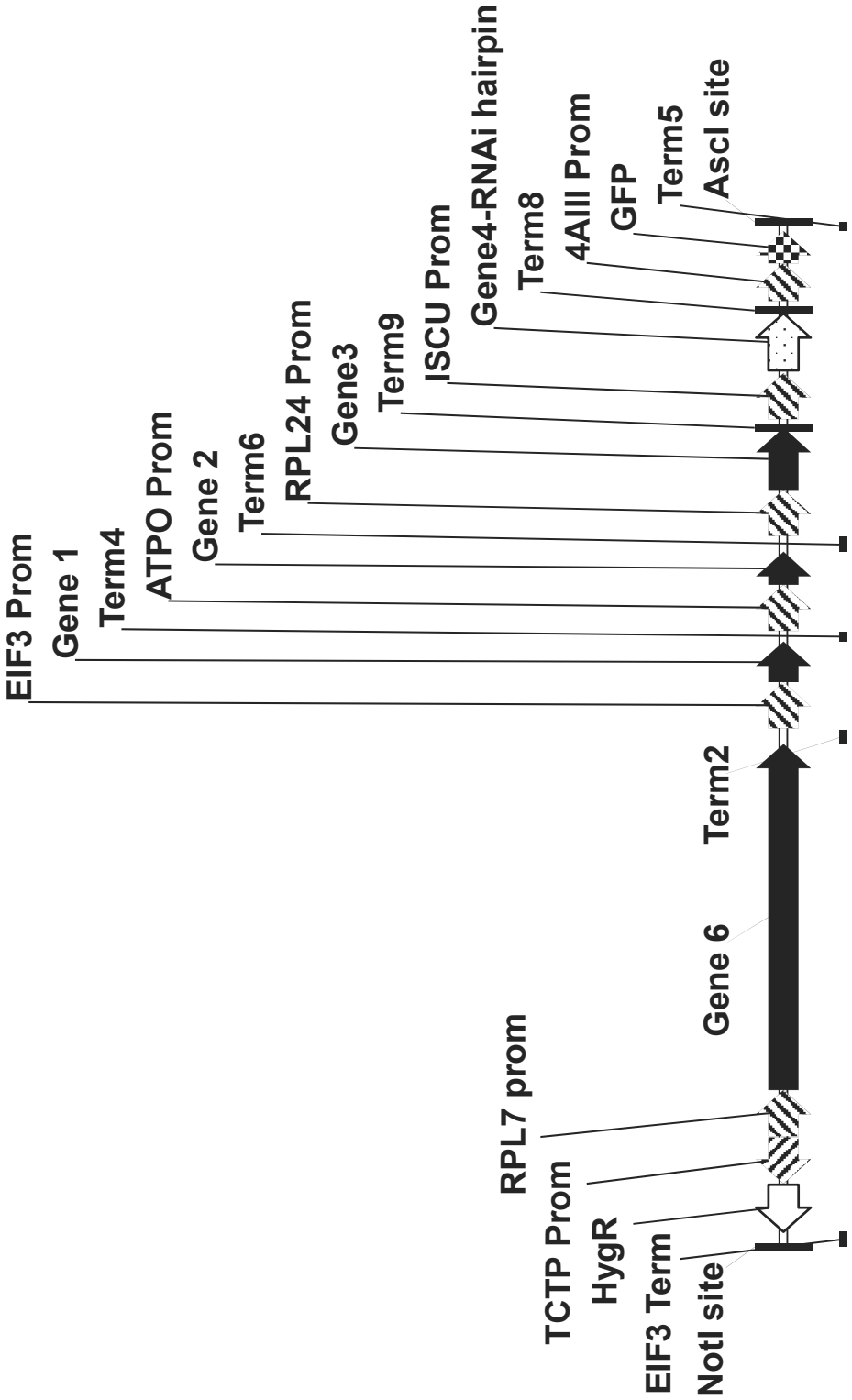

EIF3 Prom
Gene 1
Term4
ATPO Prom
Gene 2
Term6
RPL24 Prom
Gene3
Term9
ISCU Prom
Gene4-RNAi hairpin
Term8
4AIII Prom
GFP
Term5
AscI site
Term2
Gene 6
RPL7 prom
TCTP Prom
HygR
EIF3 Term
NotI site
pSGE-6337-AscNotFrag

FIG. 16

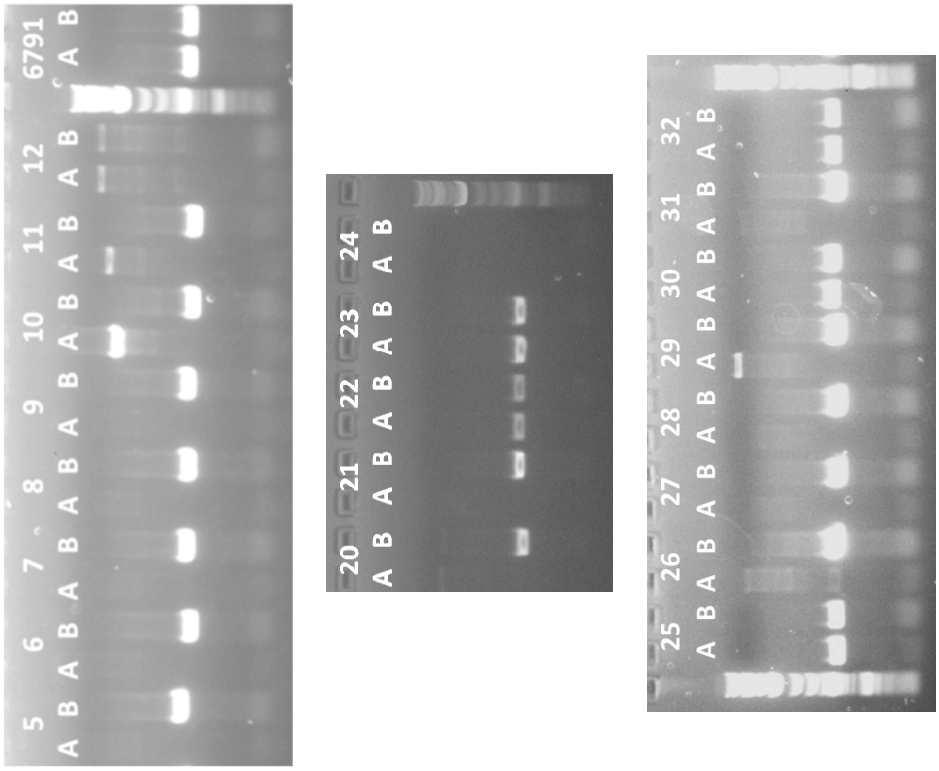

5 6 7 8 9 10 11 12 6791
A B A B A B A B A B A B A B A B
20 21 22 23 24
A B A B A B A B A B
25 26 27 28 29 30 31 32
A B A B A B A B A B A B A B A B

FIG. 17

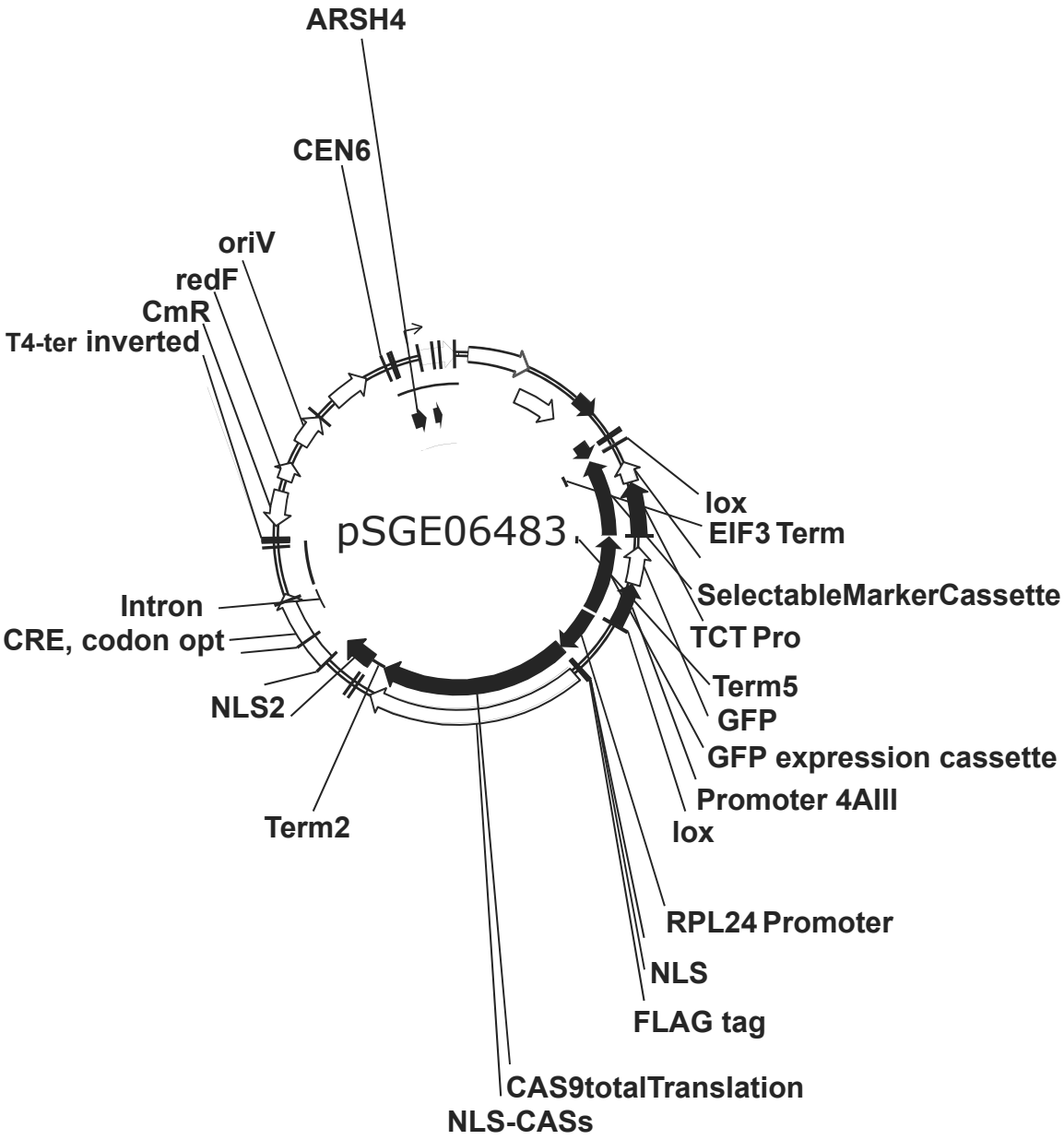
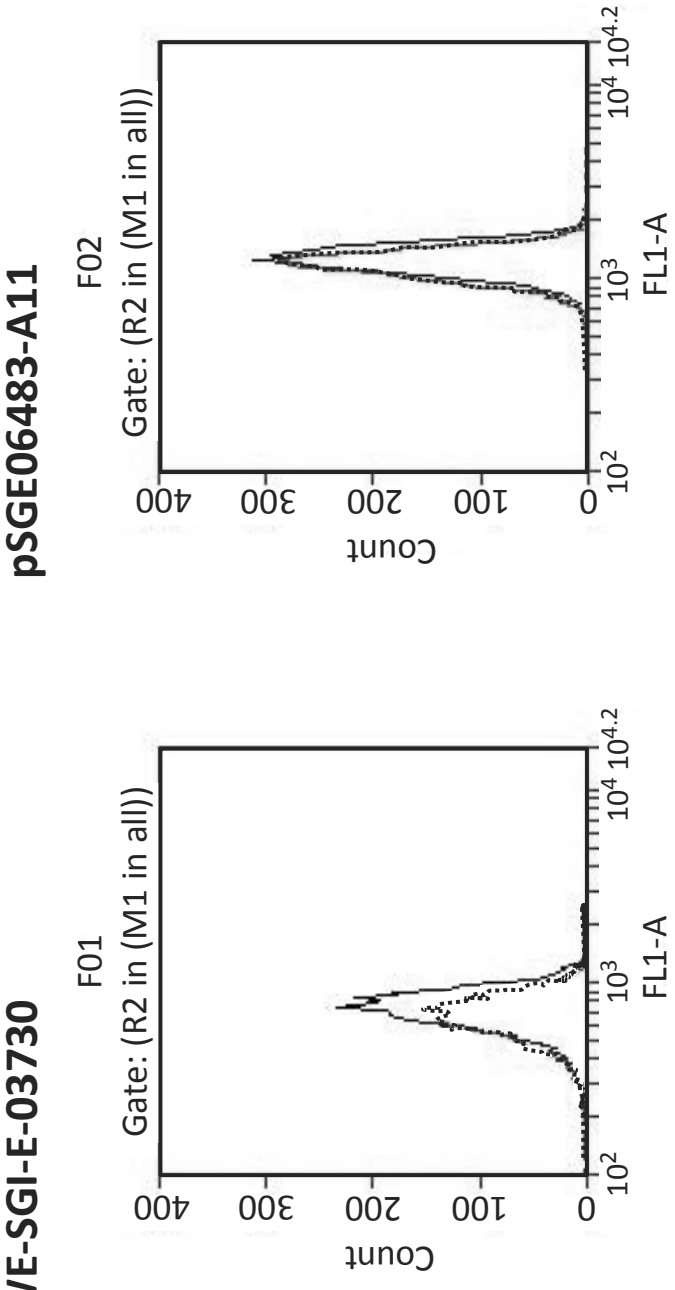
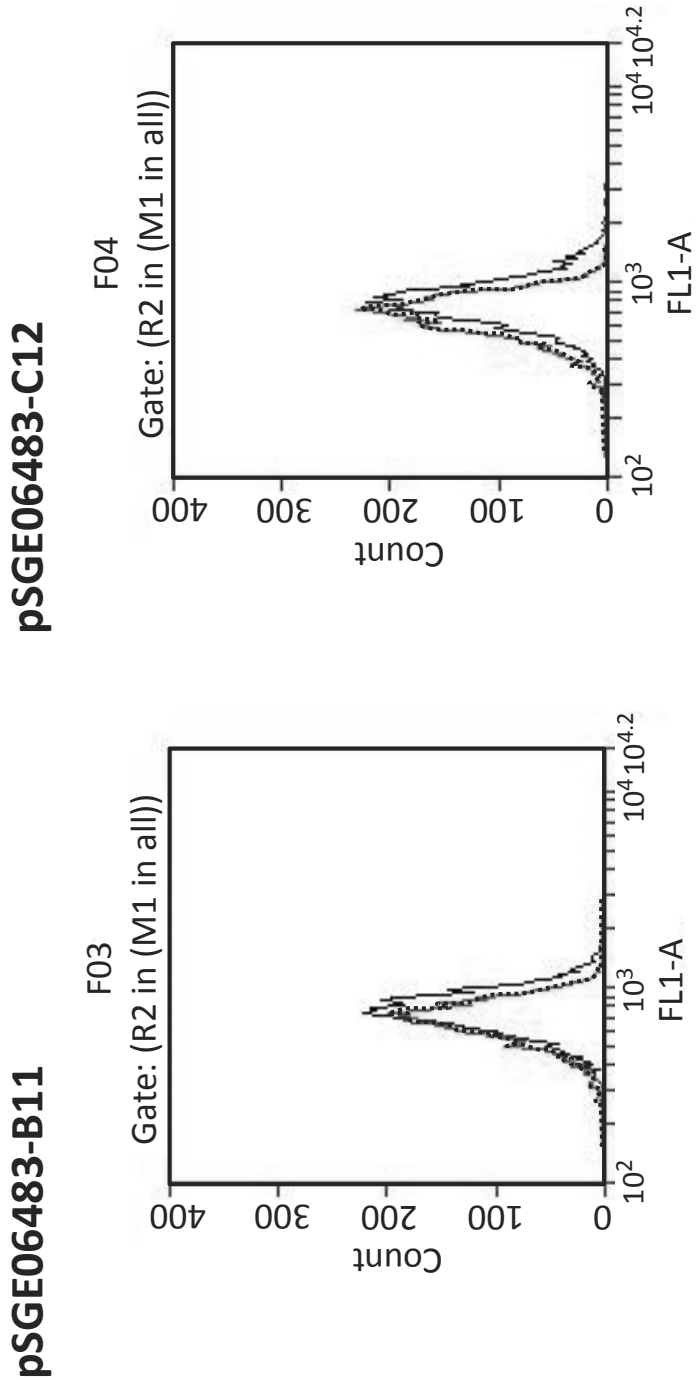

ARSH4
CEN6
oriV
redF
CmR
T4-ter inverted
pSGE06483
lox
EIF3 Term
SelectableMarkerCassette
TCT Pro
Term5
GFP
GFP expression cassette
Promoter 4AIII
lox
RPL24 Promoter
NLS
FLAG tag
CAS9totalTranslation
NLS-CASs
Term2
NLS2
Intron
CRE, codon opt

FIG. 18



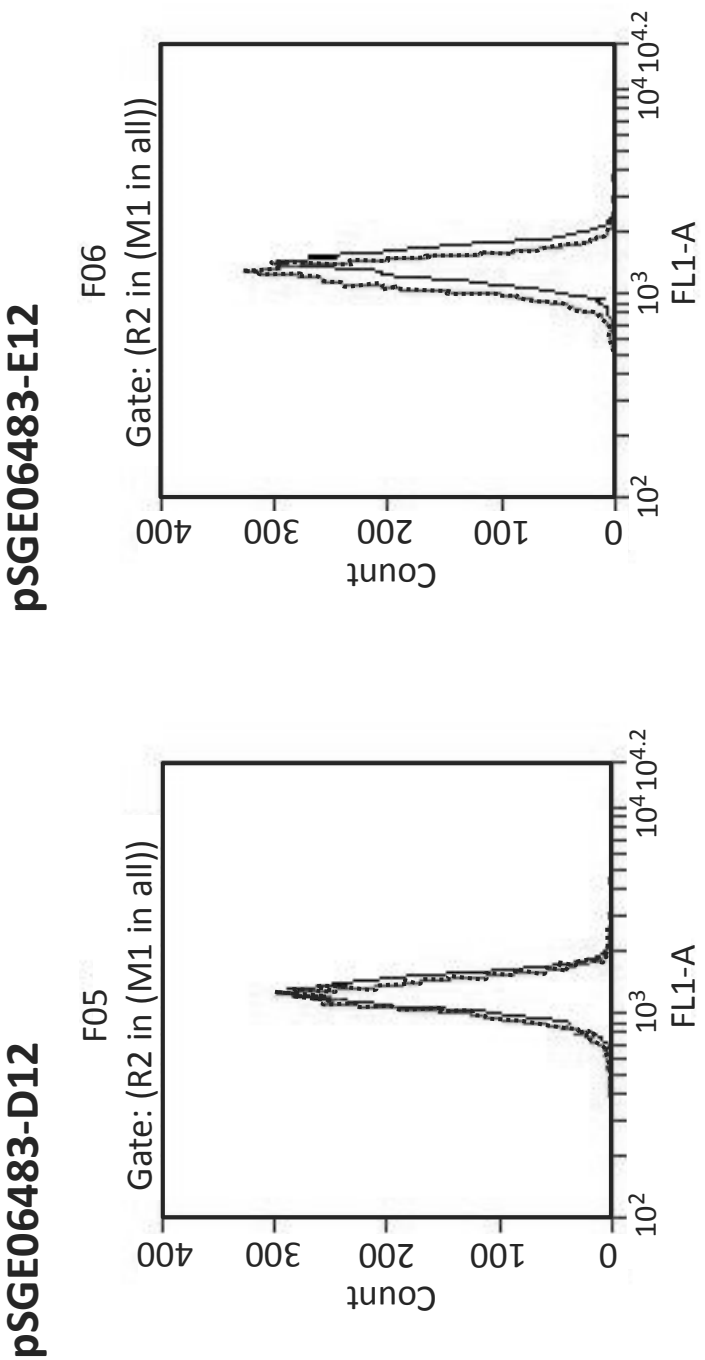
Mean Fluorescence (GFP)		
	NH_4	NO_3
WE-SGI-E-03730	728	661
pSGE06483-A11	1262	1180

FIG. 19A



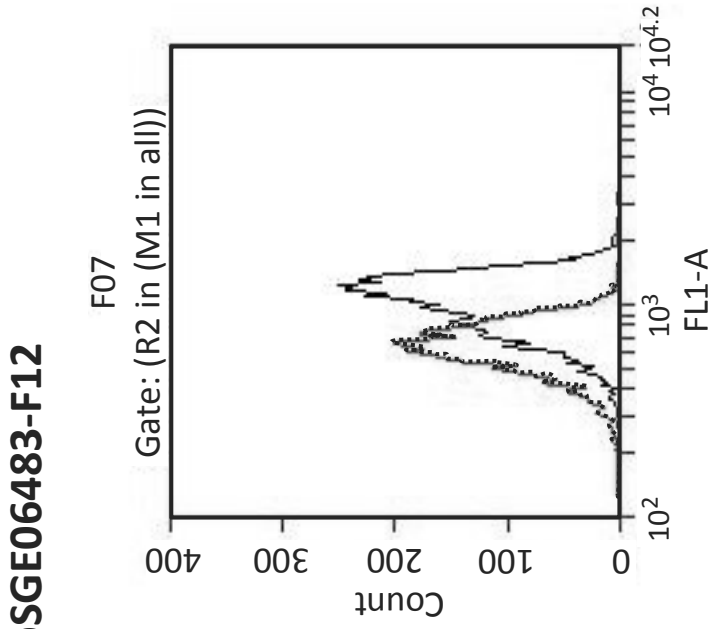
	Mean Fluorescence (GFP)	
	NH_4	NO_3
pSGE06483-B11	773	683
pSGE06483-C12	807	667

FIG. 19B



	Mean Fluorescence (GFP)	
	NH_4	NO_3
pSGE06483-D12	1266	1195
pSGE06483-E12	1415	1248

FIG. 19C



Mean Fluorescence (GFP)		
	NH_4	NO_3
pSGE06483-F12	1042	637

FIG. 19D

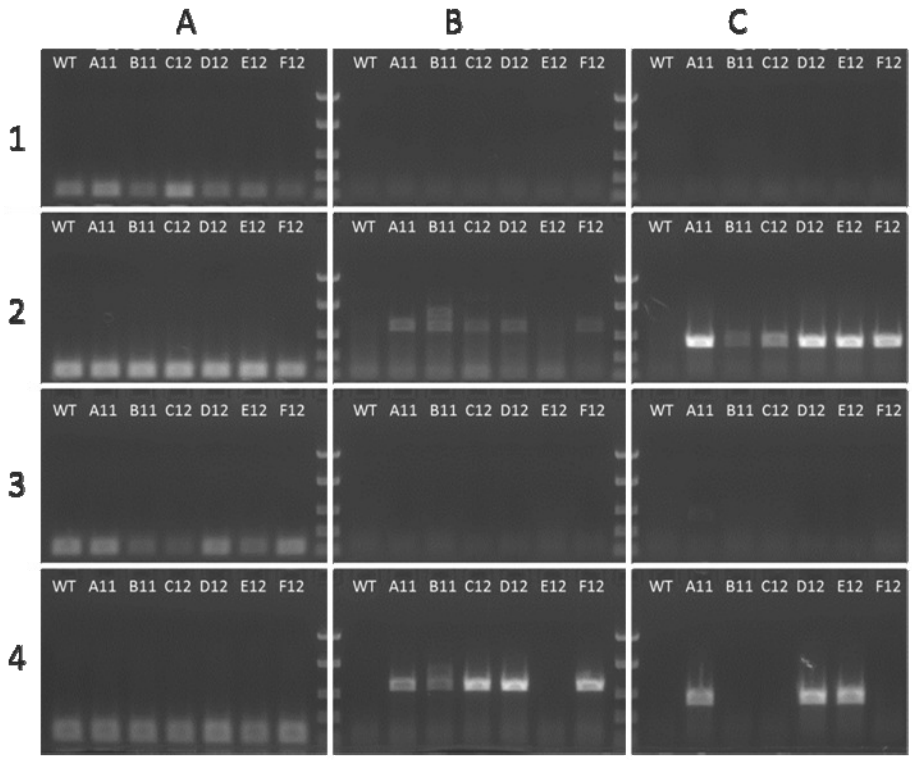


FIG. 20

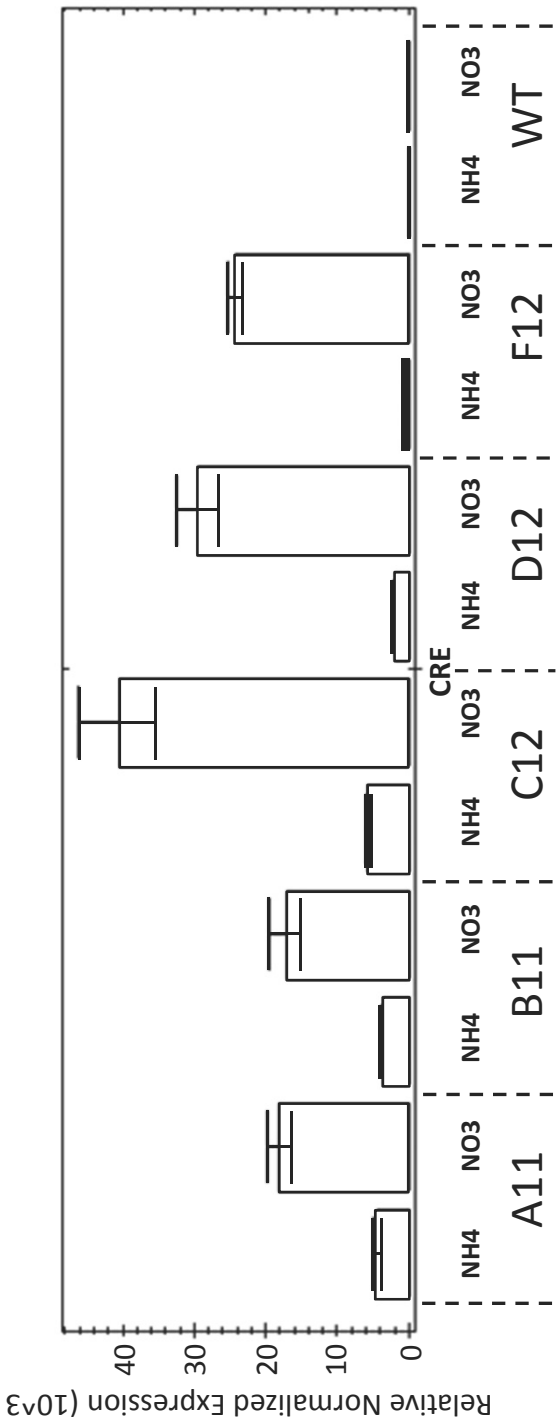

Relative Normalized Expression (10^3)
0
10
20
30
40
NH4
NO3
A11
NH4
NO3
B11
NH4
NO3
C12
CRE
NH4
NO3
D12
NH4
NO3
F12
NH4
NO3
WT

FIG. 21

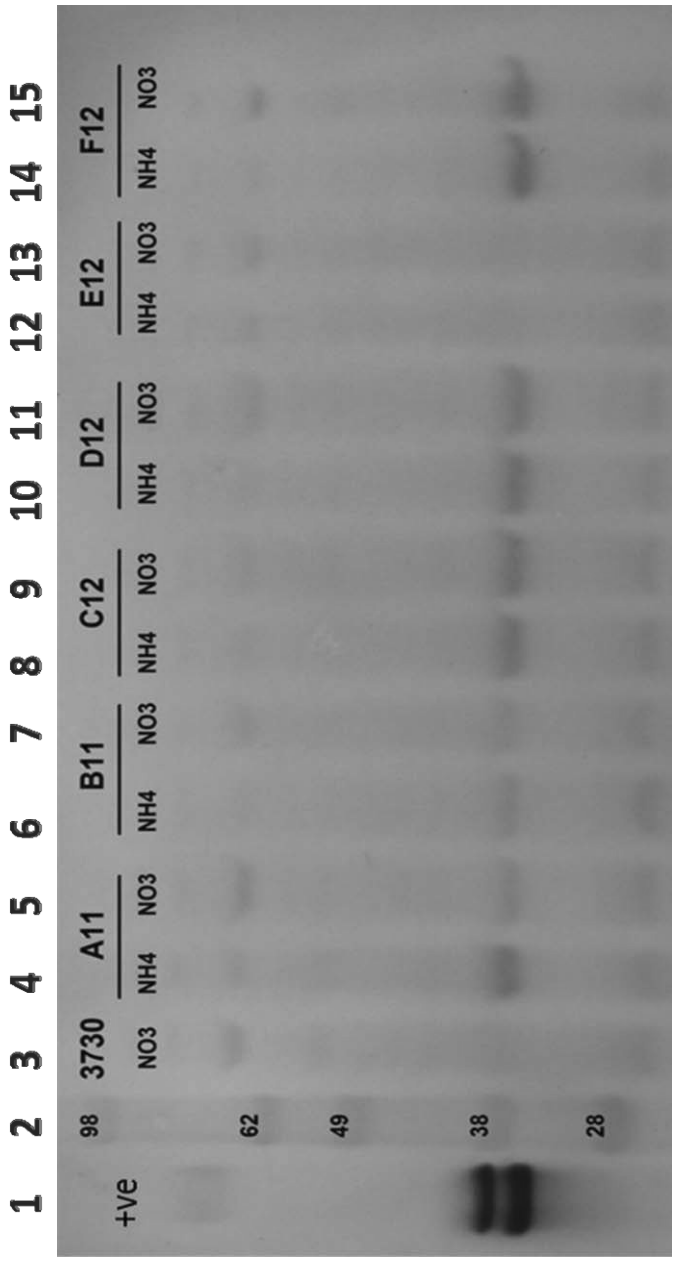

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
+Ve
98
62
49
38
28
3730
NO3
A11
NH4
NO3
B11
NH4
NO3
C12
NH4
NO3
D12
NH4
NO3
E12
NH4
NO3
F12
NH4
NO3

FIG. 22

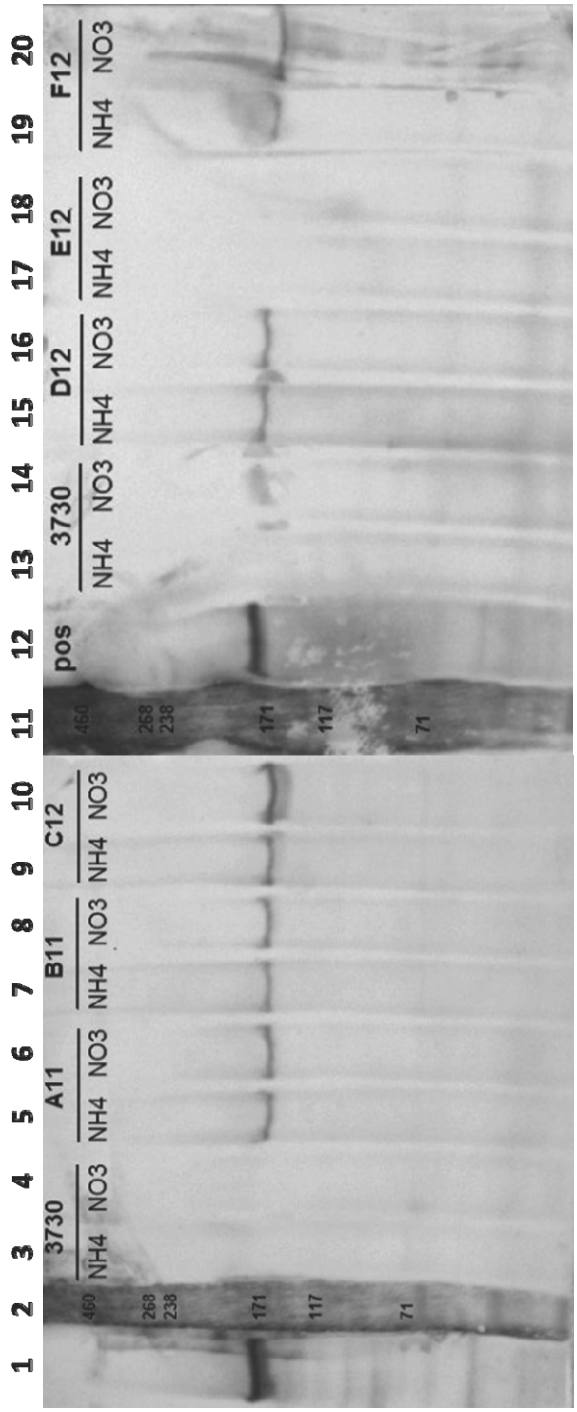

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
3730 NH4 NO3
A11 NH4 NO3
B11 NH4 NO3
C12 NH4 NO3
pos
3730 NH4 NO3
D12 NH4 NO3
E12 NH4 NO3
F12 NH4 NO3
460
268
238
171
117
71

FIG. 23

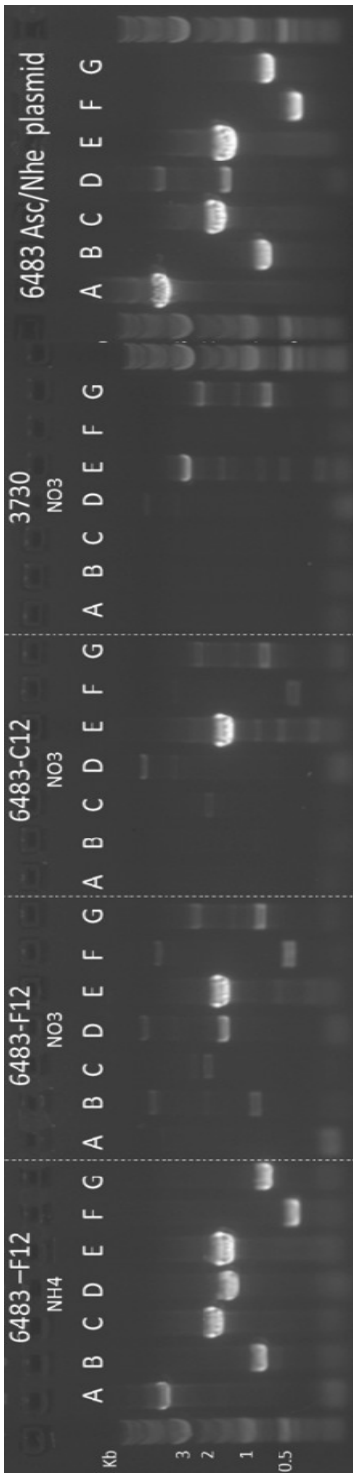

6483 –F12
NH4
6483-F12
NO3
6483-C12
NO3
3730
NO3
6483 Asc/Nhe plasmid
A B C D E F G
Kb
3
2
1
0.5

FIG. 24

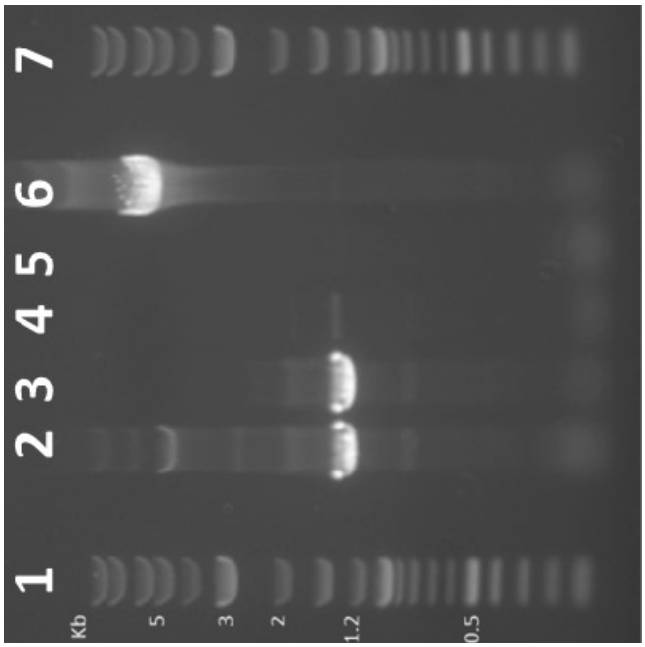


FIG. 25

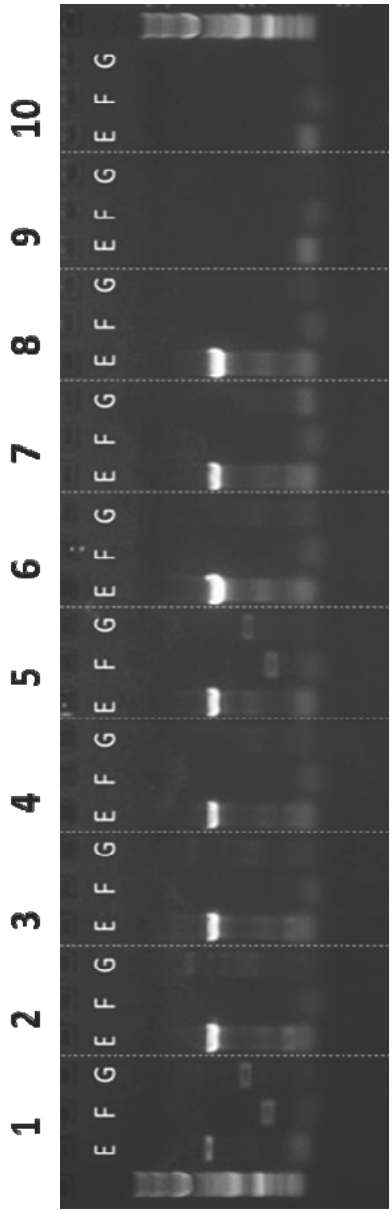


FIG. 26

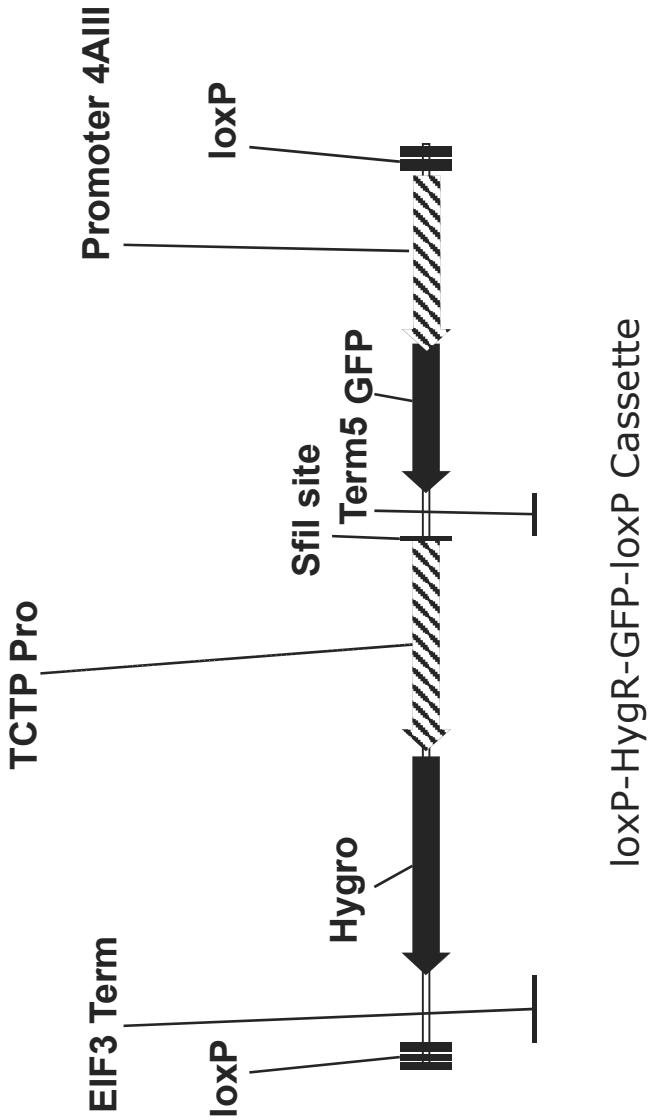

Promoter 4AIII
loxP
Term5 GFP
SfiI site
TCTP Pro
Hygro
EIF3 Term
loxP
loxP-HygR-GFP-loxP Cassette

FIG. 27

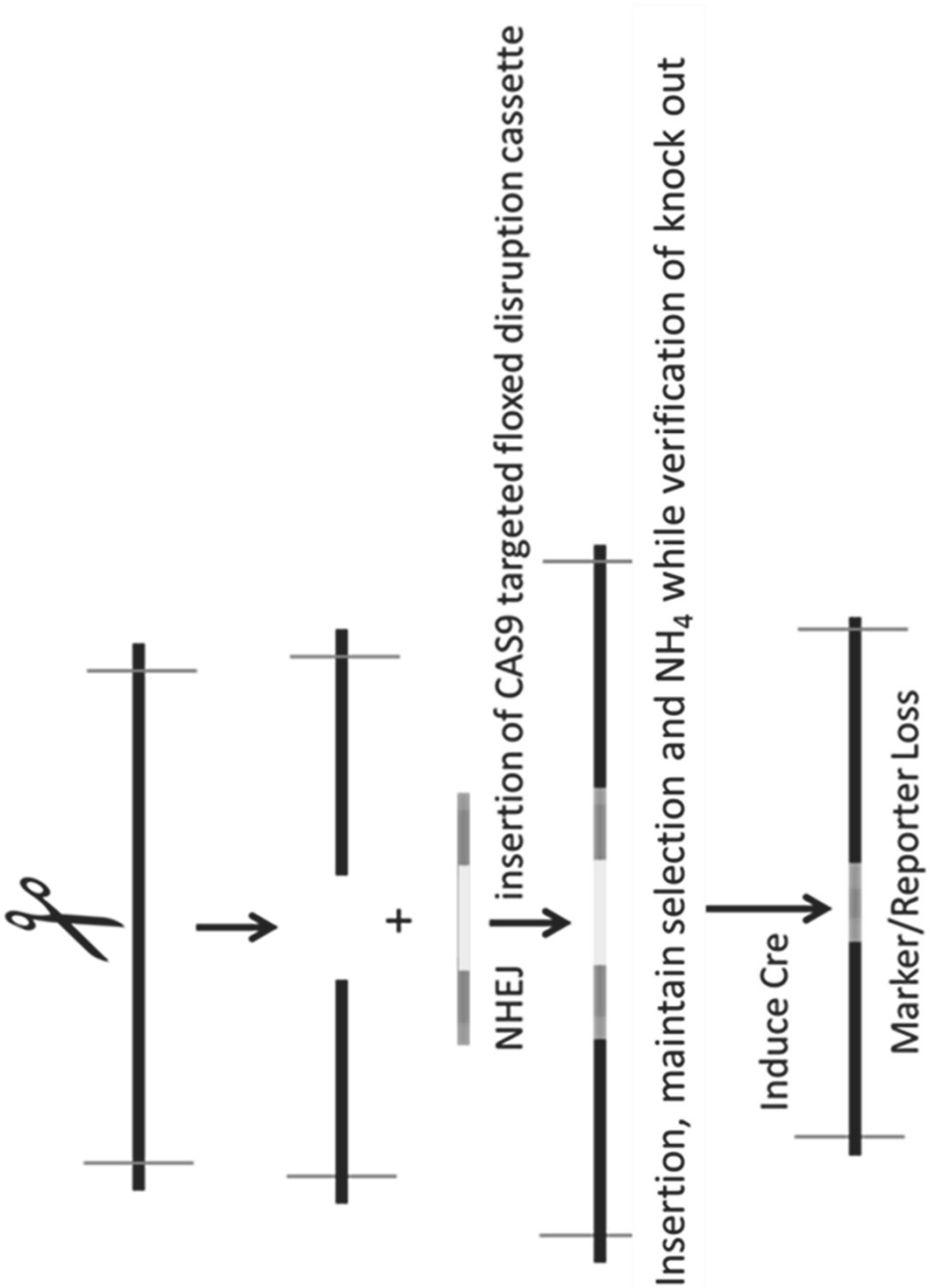

NHEJ
insertion of CAS9 targeted floxed disruption cassette
Insertion, maintain selection and NH4 while verification of knock out
Induce Cre
Marker/Reporter Loss

FIG. 28

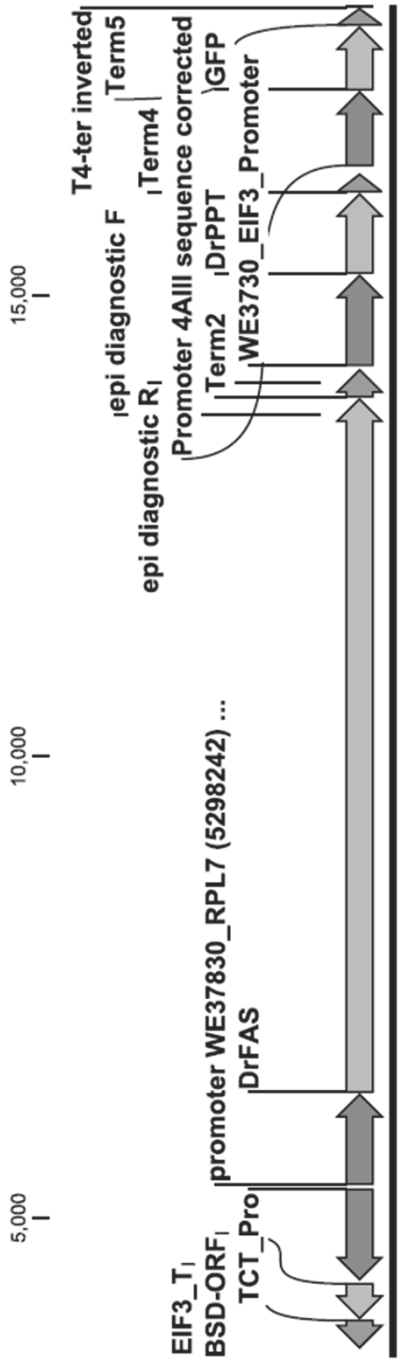

5,000
10,000
15,000
EIF3_T
BSD-ORF
TCT_Pro
promoter WE37830_RPL7 (5298242) ...
DrFAS
epi diagnostic R
epi diagnostic F
Promoter 4AIII sequence corrected
Term2
DrPPT
WE3730_EIF3_Promoter
GFP
Term4
T4-ter inverted
Term5

FIG. 29

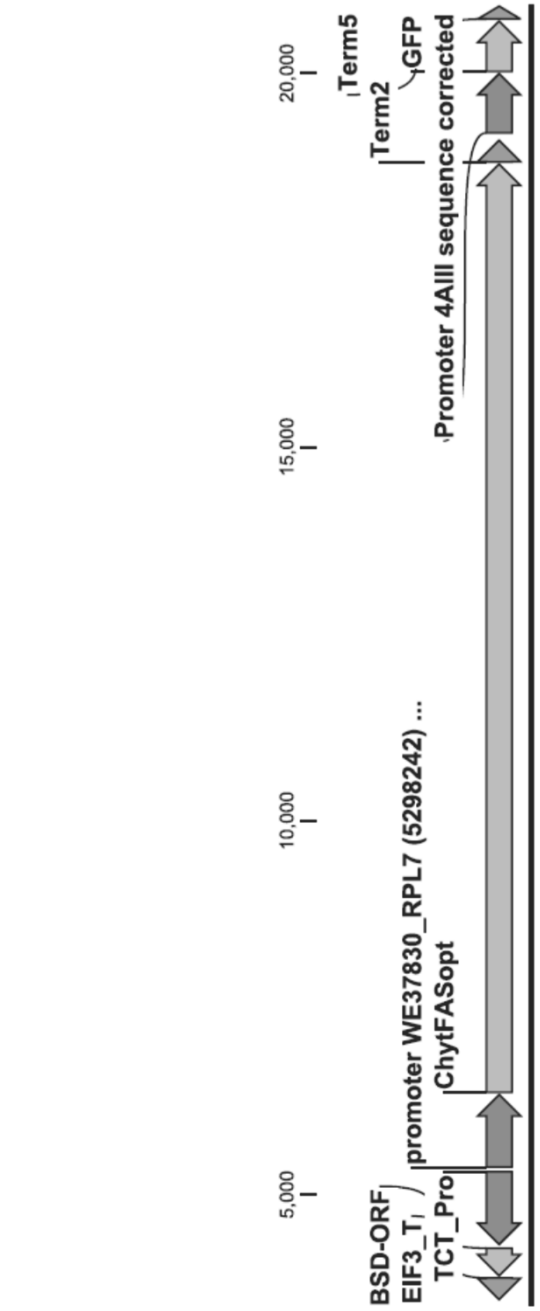

5,000
10,000
15,000
20,000
BSD-ORF
EIF3_T
TCT_Pro
promoter WE37830_RPL7 (5298242) ...
ChytFASopt
Promoter 4AIII sequence corrected
Term2
Term5
GFP

FIG. 30

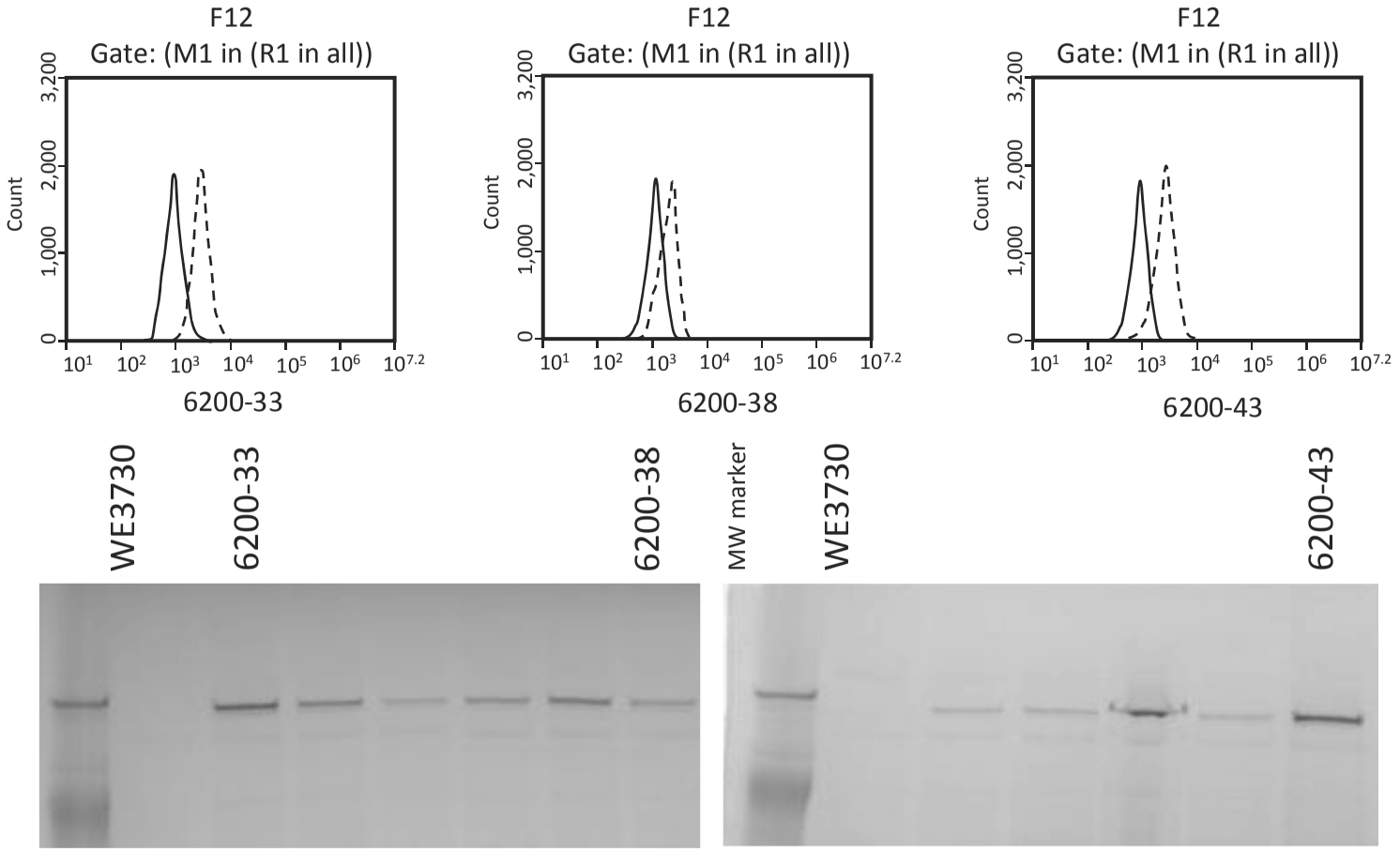

F12
Gate: (M1 in (R1 in all))
Count
3,200
2,000
1,000
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
6200-33
F12
Gate: (M1 in (R1 in all))
Count
3,200
2,000
1,000
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
6200-38
F12
Gate: (M1 in (R1 in all))
Count
3,200
2,000
1,000
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
6200-43
WE3730
6200-33
6200-38
MW marker
WE3730
6200-43
α-AnimalFAS

FIG. 31A

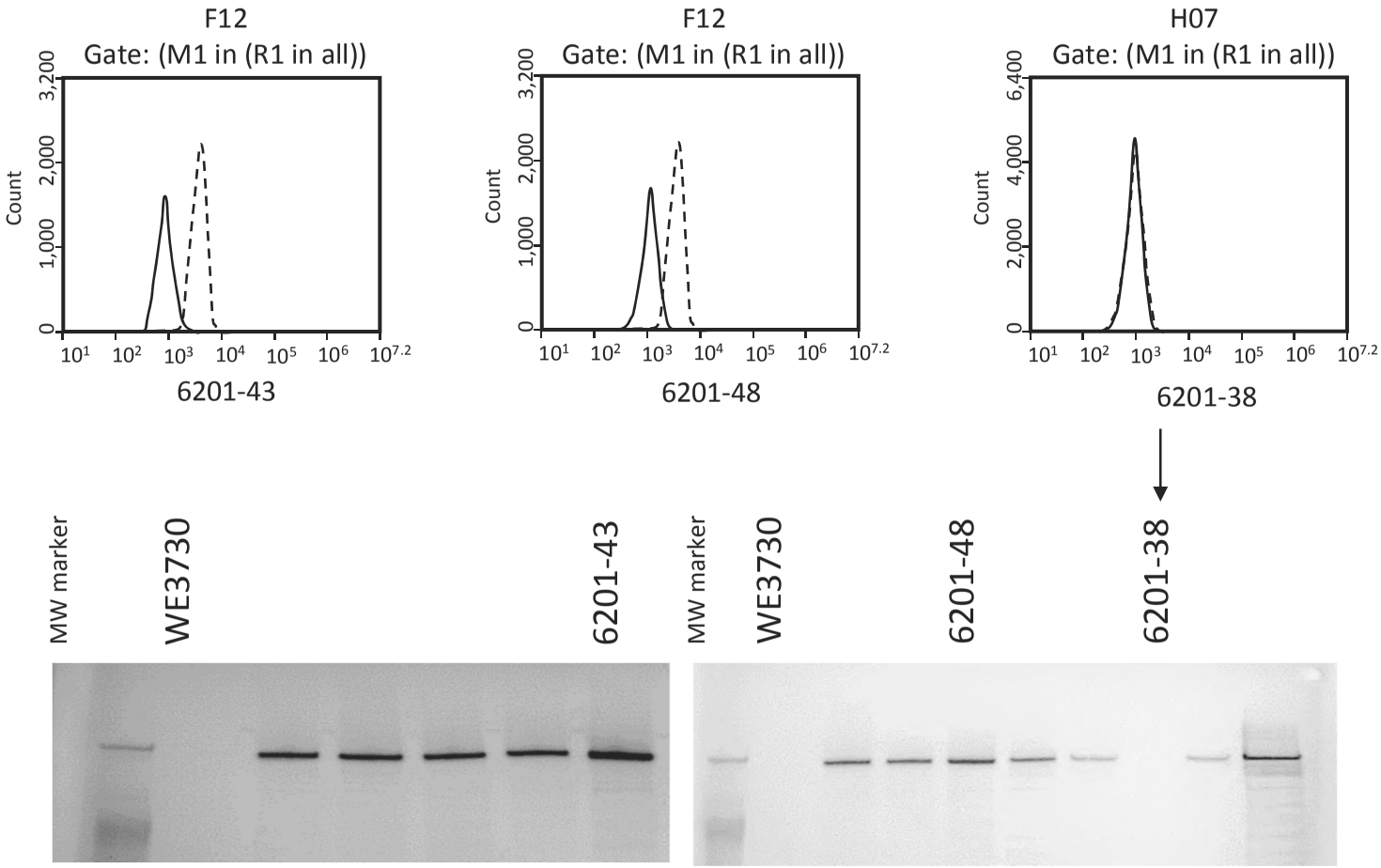

F12
Gate: (M1 in (R1 in all))
Count
3,200
2,000
1,000
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
6201-43
F12
Gate: (M1 in (R1 in all))
Count
3,200
2,000
1,000
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
6201-48
H07
Gate: (M1 in (R1 in all))
Count
6,400
4,000
2,000
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
6201-38
MW marker
WE3730
6201-43
MW marker
WE3730
6201-48
6201-38
α-FLAG

FIG. 31B

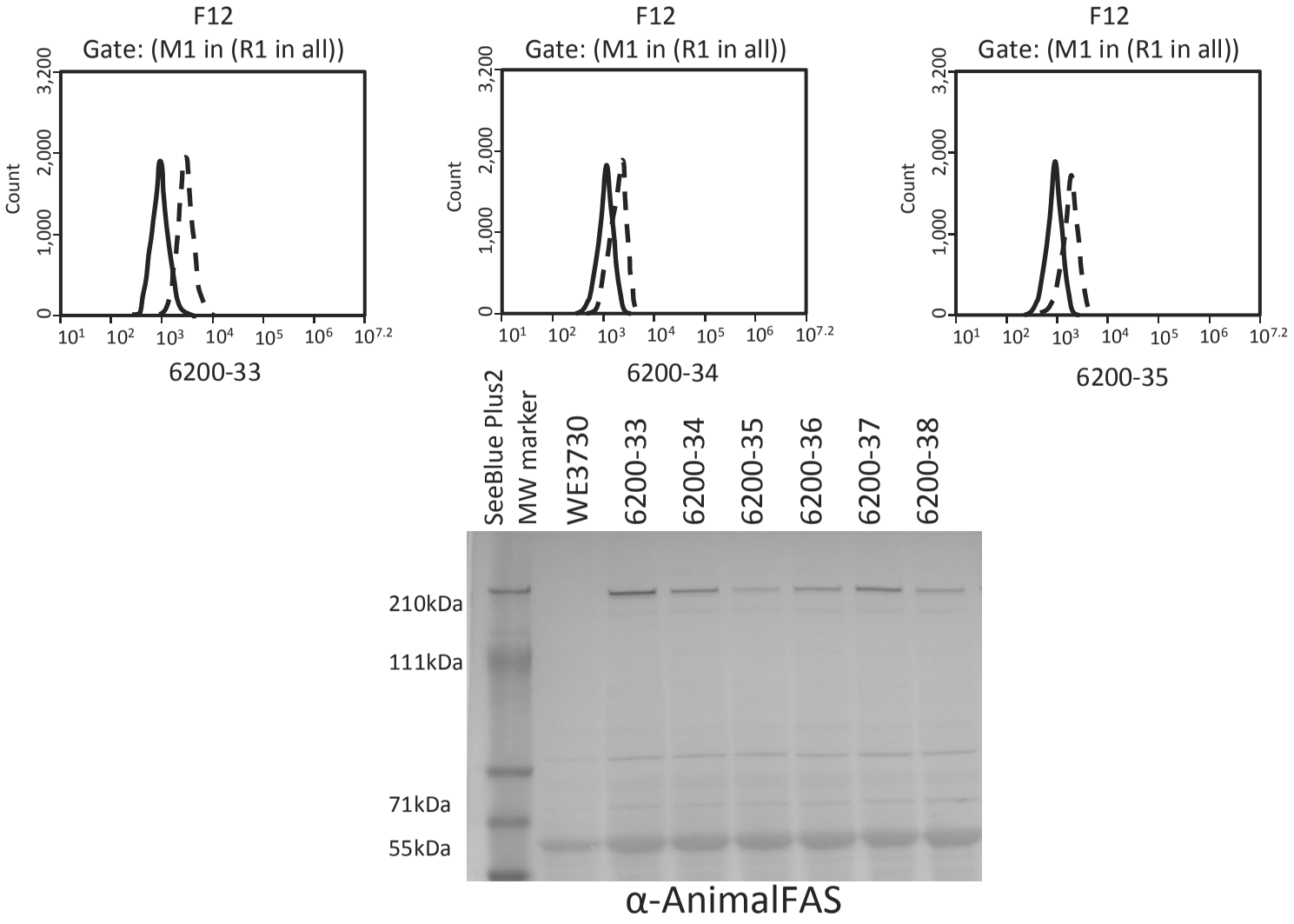

F12
Gate: (M1 in (R1 in all))
Count
3,200
2,000
1,000
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
6200-33
F12
Gate: (M1 in (R1 in all))
Count
3,200
2,000
1,000
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
6200-34
F12
Gate: (M1 in (R1 in all))
Count
3,200
2,000
1,000
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
6200-35
SeeBlue Plus2
MW marker
WE3730
6200-33
6200-34
6200-35
6200-36
6200-37
6200-38
210kDa
111kDa
71kDa
55kDa
α-AnimalFAS

FIG. 32A

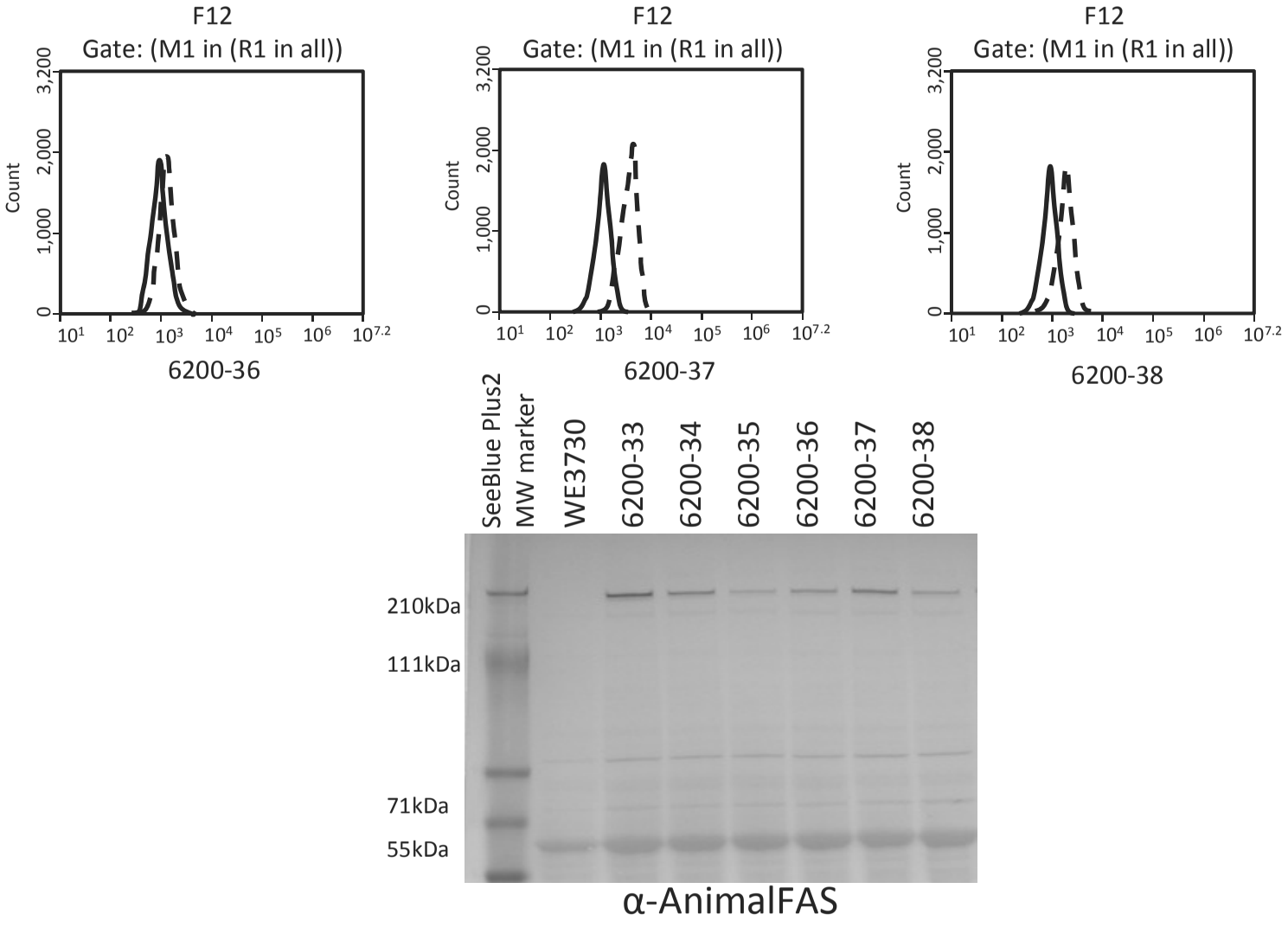
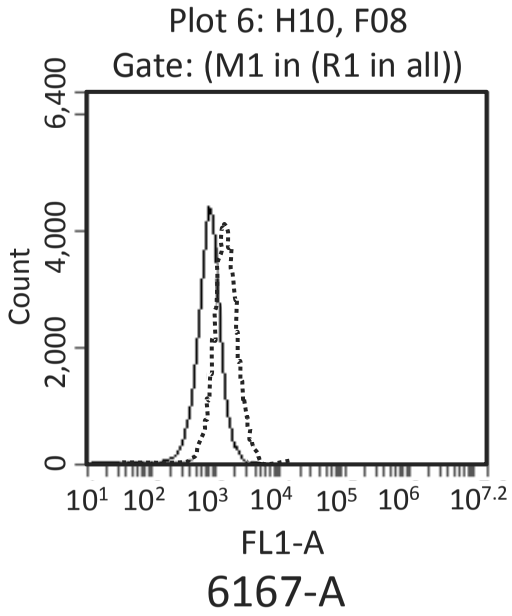
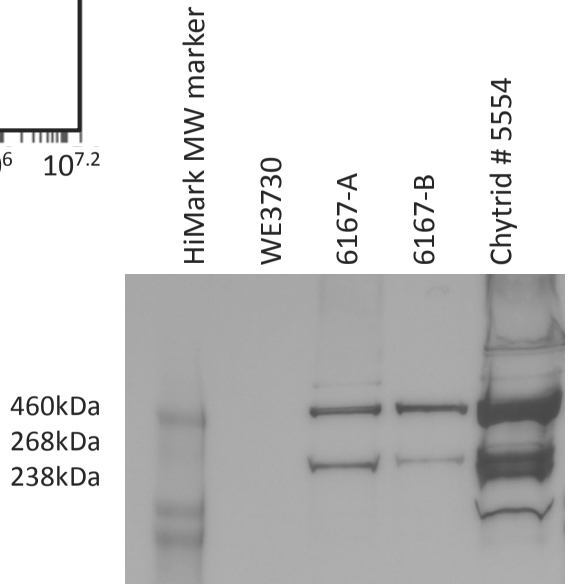

F12
Gate: (M1 in (R1 in all))
Count
3,200
2,000
1,000
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
6200-36
F12
Gate: (M1 in (R1 in all))
Count
3,200
2,000
1,000
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
6200-37
F12
Gate: (M1 in (R1 in all))
Count
3,200
2,000
1,000
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
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

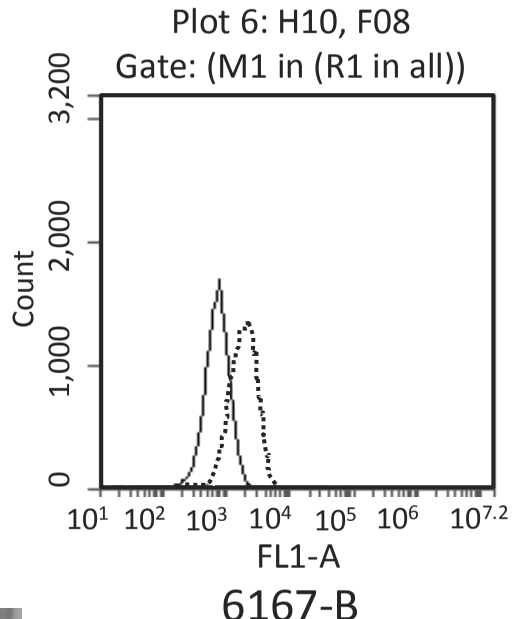
FIG. 32B



6167-A



α-ChytridFAS



6167-B

FIG. 33

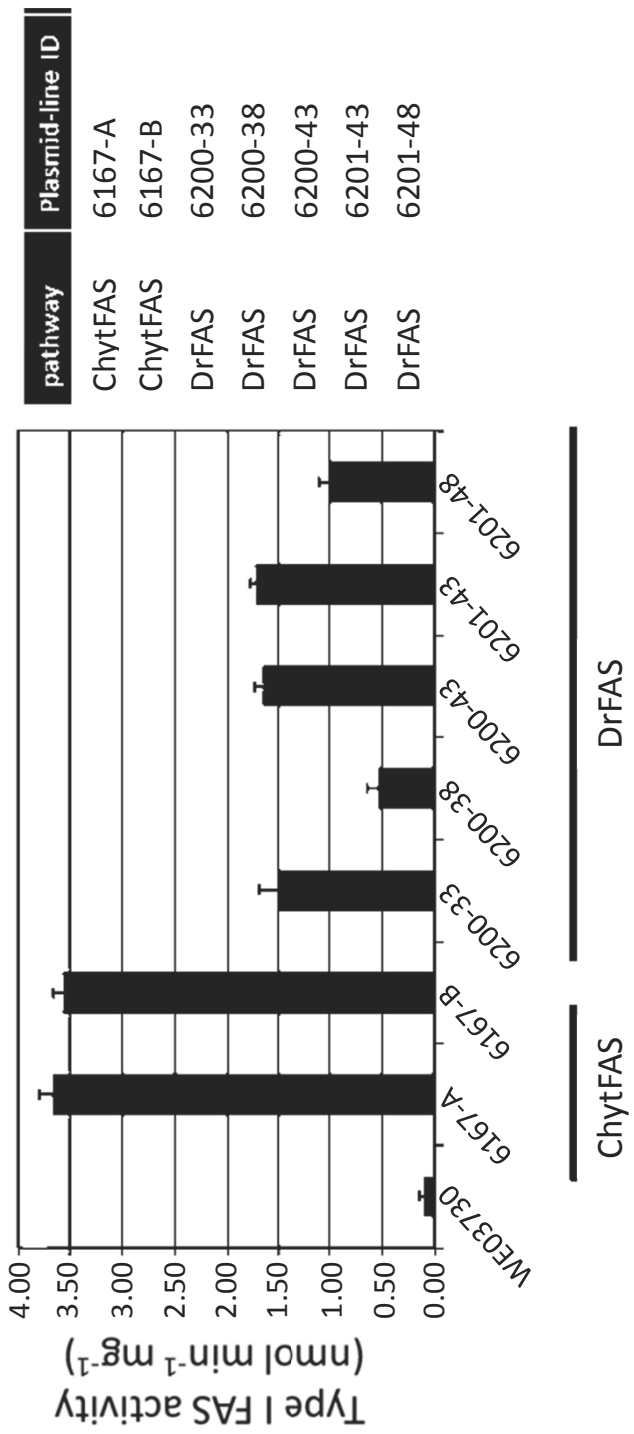
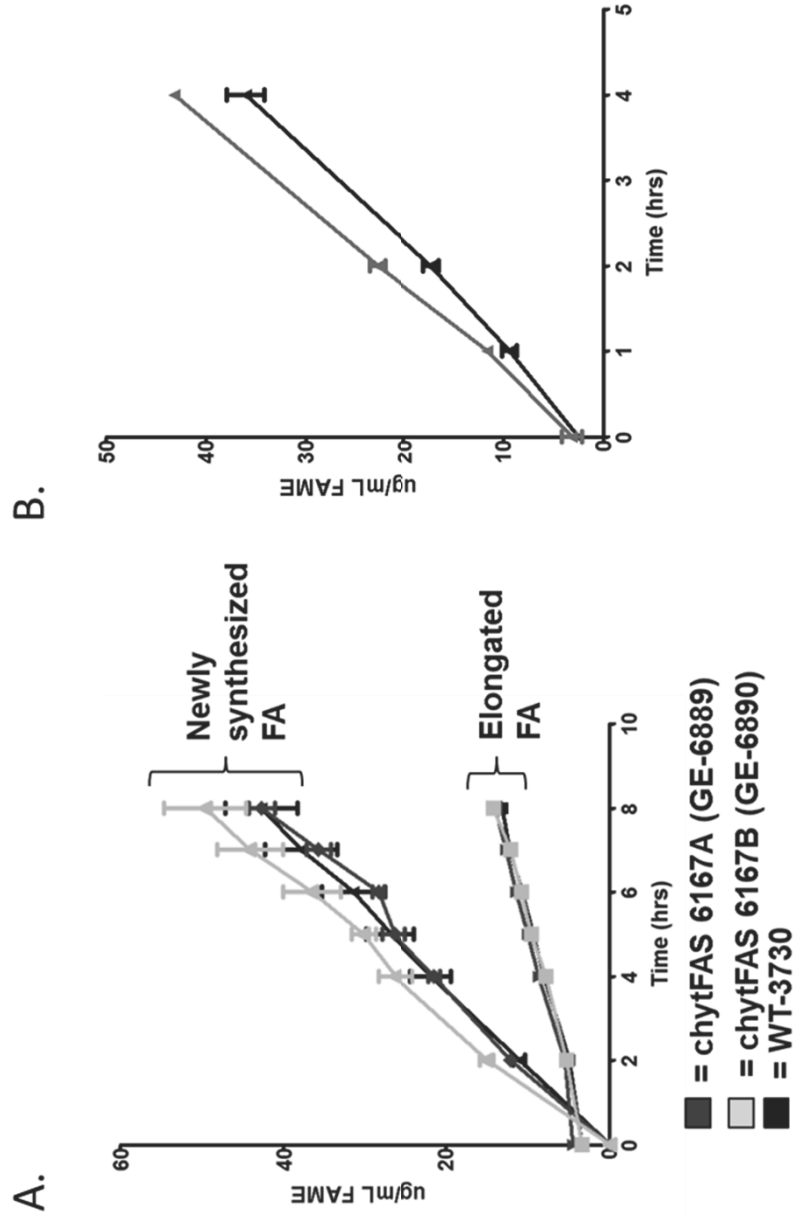
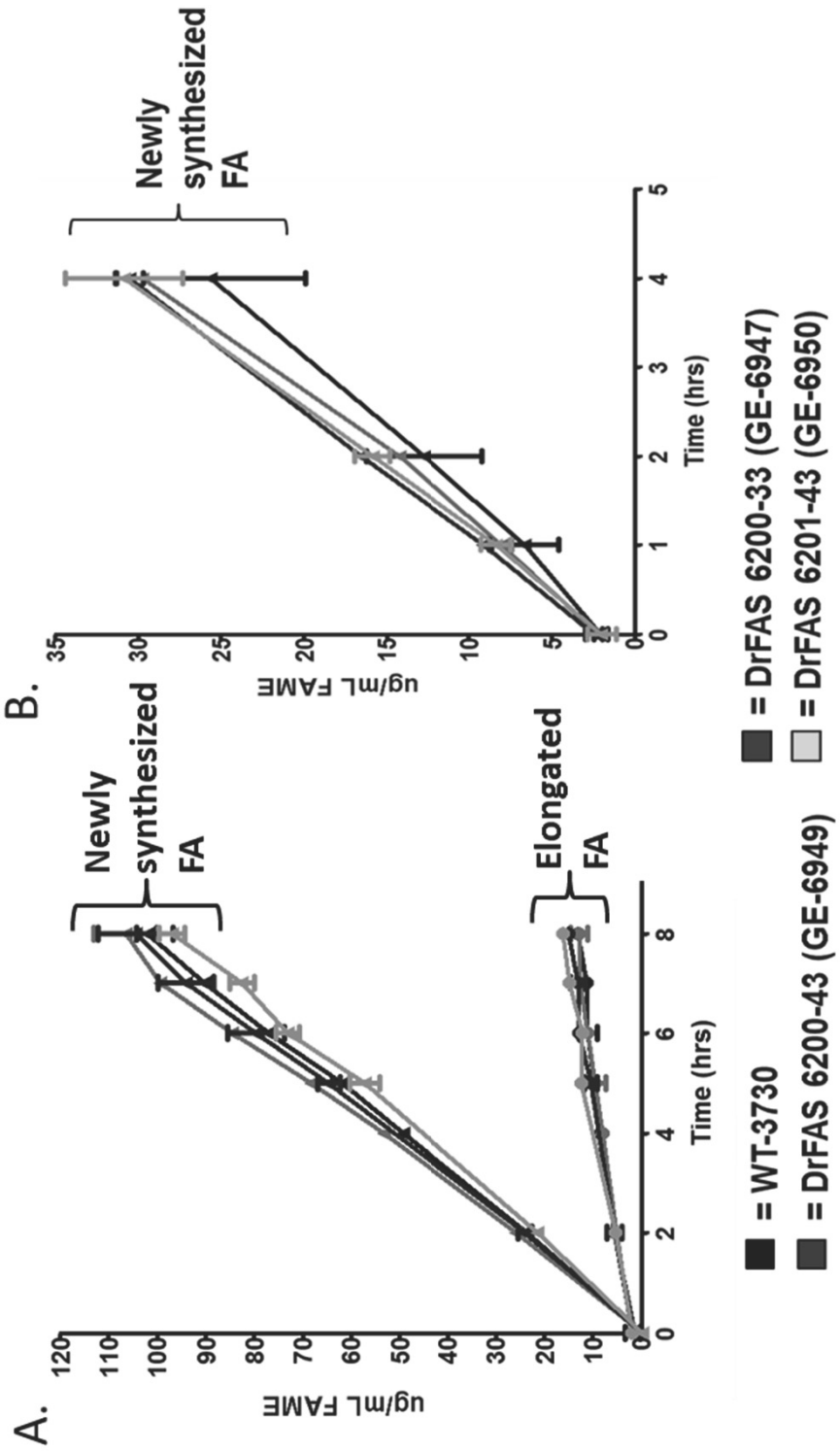

Type I FAS activity
(nmol min-1 mg-1)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
WE03730
6167-A
6167-B
6200-33
6200-38
6200-43
6201-43
6201-48
ChytFAS
DrFAS
pathway
Plasmid-line ID
ChytFAS 6167-A
ChytFAS 6167-B
DrFAS 6200-33
DrFAS 6200-38
DrFAS 6200-43
DrFAS 6201-43
DrFAS 6201-48

FIG. 34


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

FIGS. 35A-B


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

FIGS. 36A-B