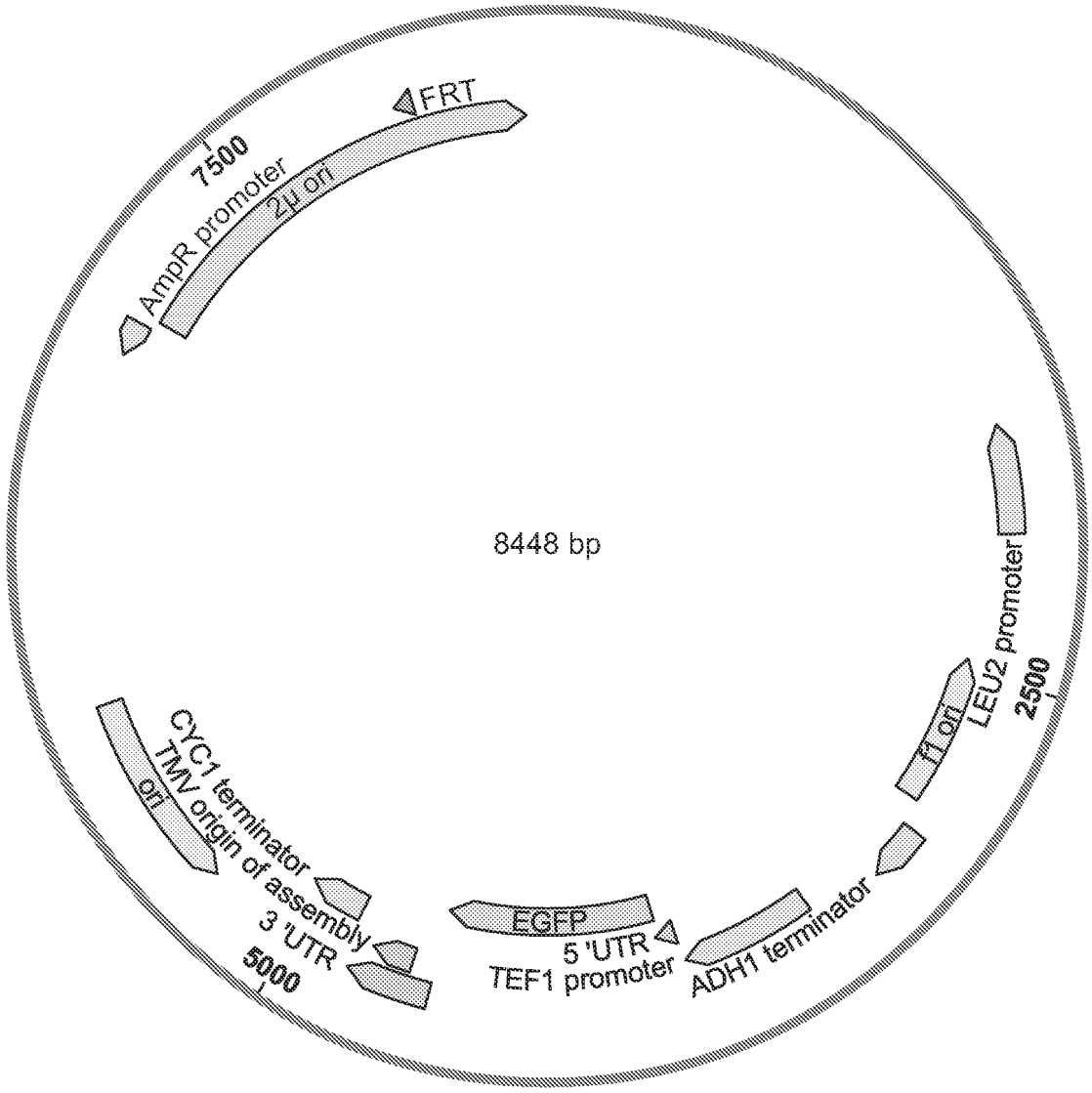

FRT
7500
AmpR promoter
2μ ori
8448 bp
f1 ori
LEU2 promoter
2500
CYC1 terminator
TMV origin of assembly
ori
3 'UTR
5000
EGFP
5 'UTR
TEF1 promoter
ADH1 terminator

FIG. 1

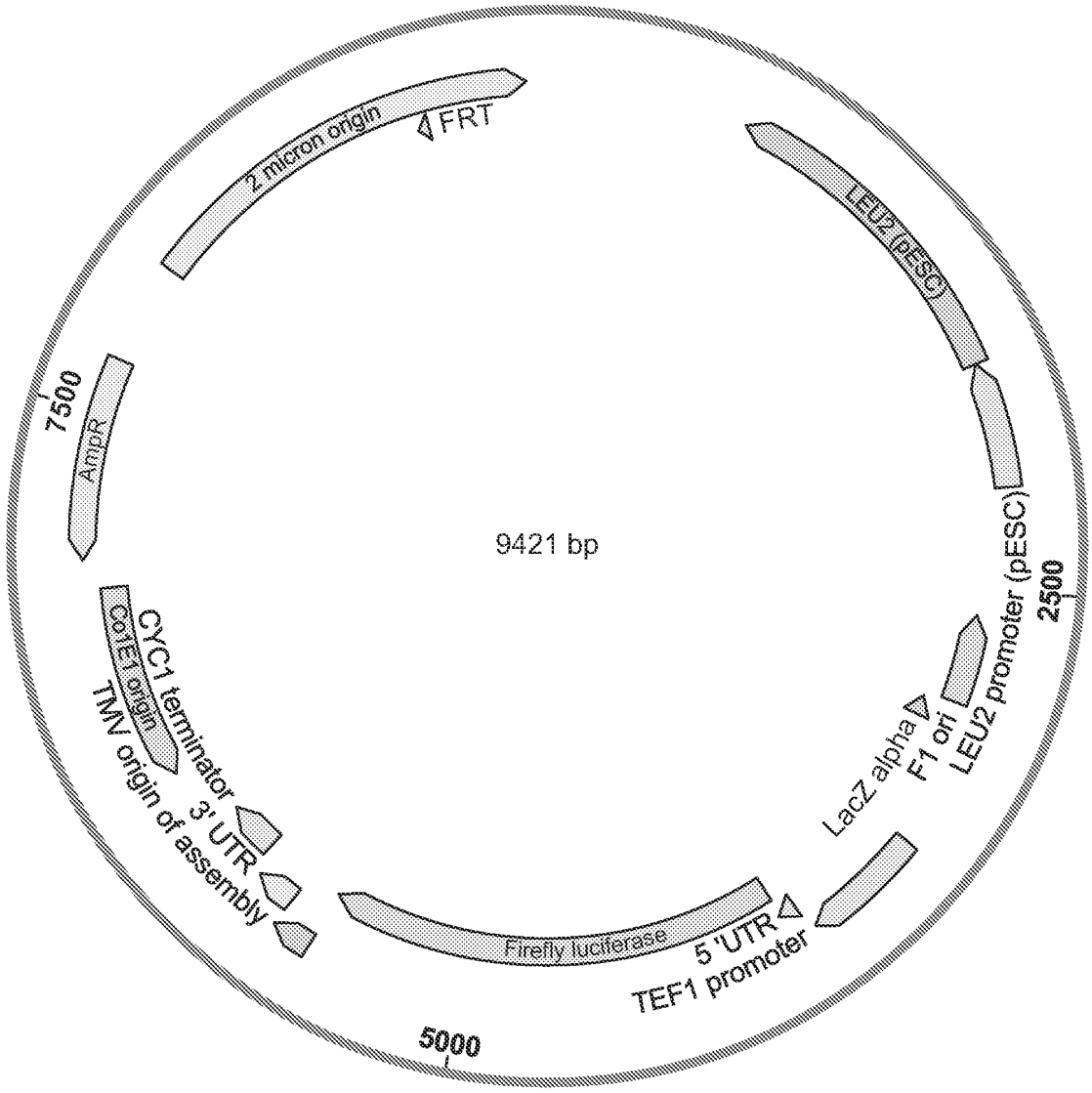

2 micron origin
FRT
LEU2 (pESC)
LEU2 promoter (pESC)
F1 ori
LacZ alpha
5 'UTR
TEF1 promoter
Firefly luciferase
3' UTR
TMV origin of assembly
CYC1 terminator
ColE1 origin
AmpR
9421 bp
2500
5000
7500

FIG. 2

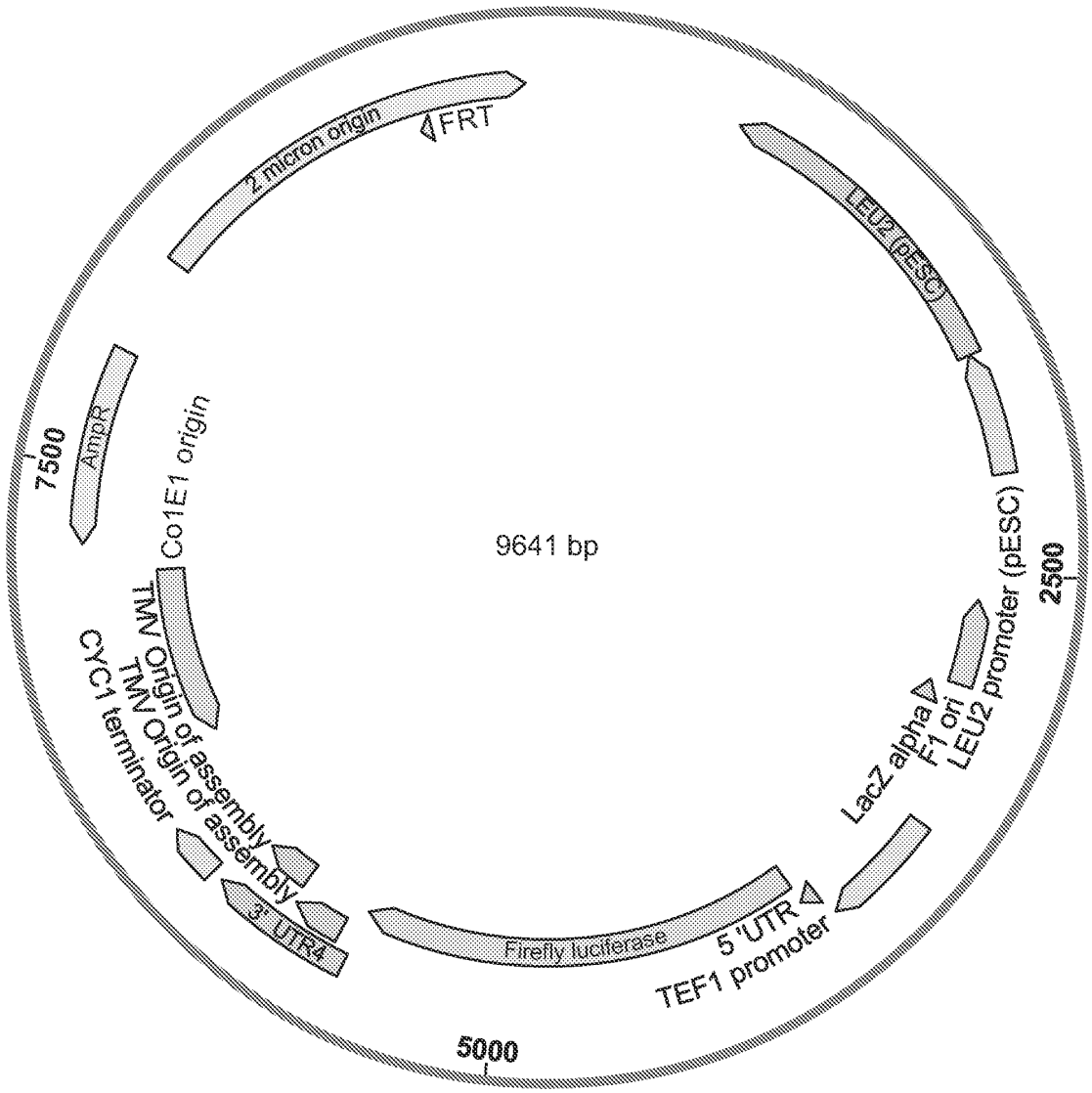

2 micron origin
FRT
LEU2 (pESC)
AmpR
7500
Co1E1 origin
9641 bp
2500
TMV Origin of assembly
CYC1 terminator
TMV Origin of assembly
LacZ alpha
F1 ori
LEU2 promoter (pESC)
3' UTR4
Firefly luciferase
5'UTR
TEF1 promoter
5000

FIG. 3

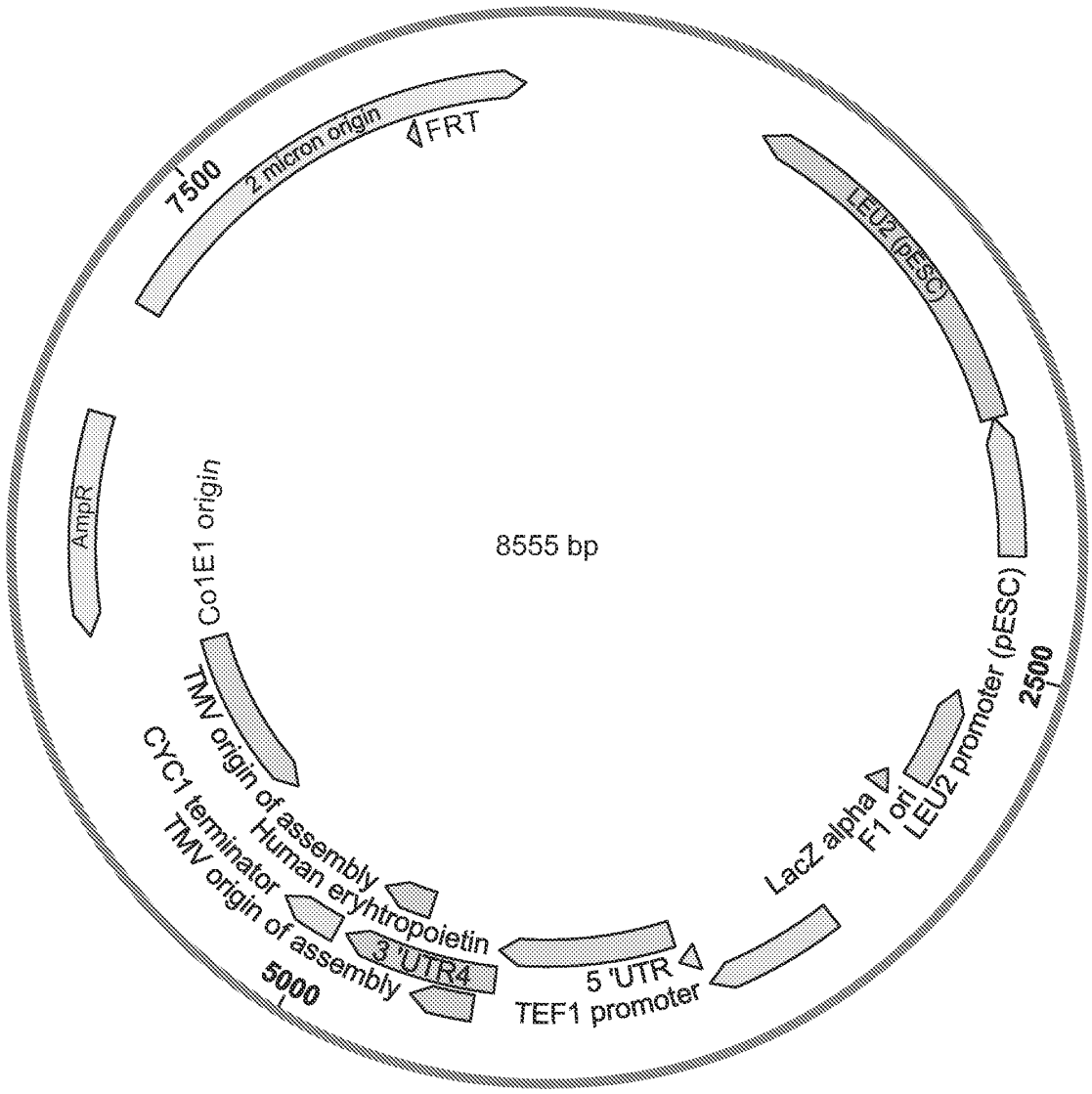

7500
2 micron origin
FRT
LEU2 (pESC)
AmpR
Co1E1 origin
8555 bp
TMV origin of assembly
Human eryhtropoietin
CYC1 terminator
TMV origin of assembly
3 'UTR4
5000
5 'UTR
TEF1 promoter
LacZ alpha
F1 ori
LEU2 promoter (pESC)
2500

FIG. 4

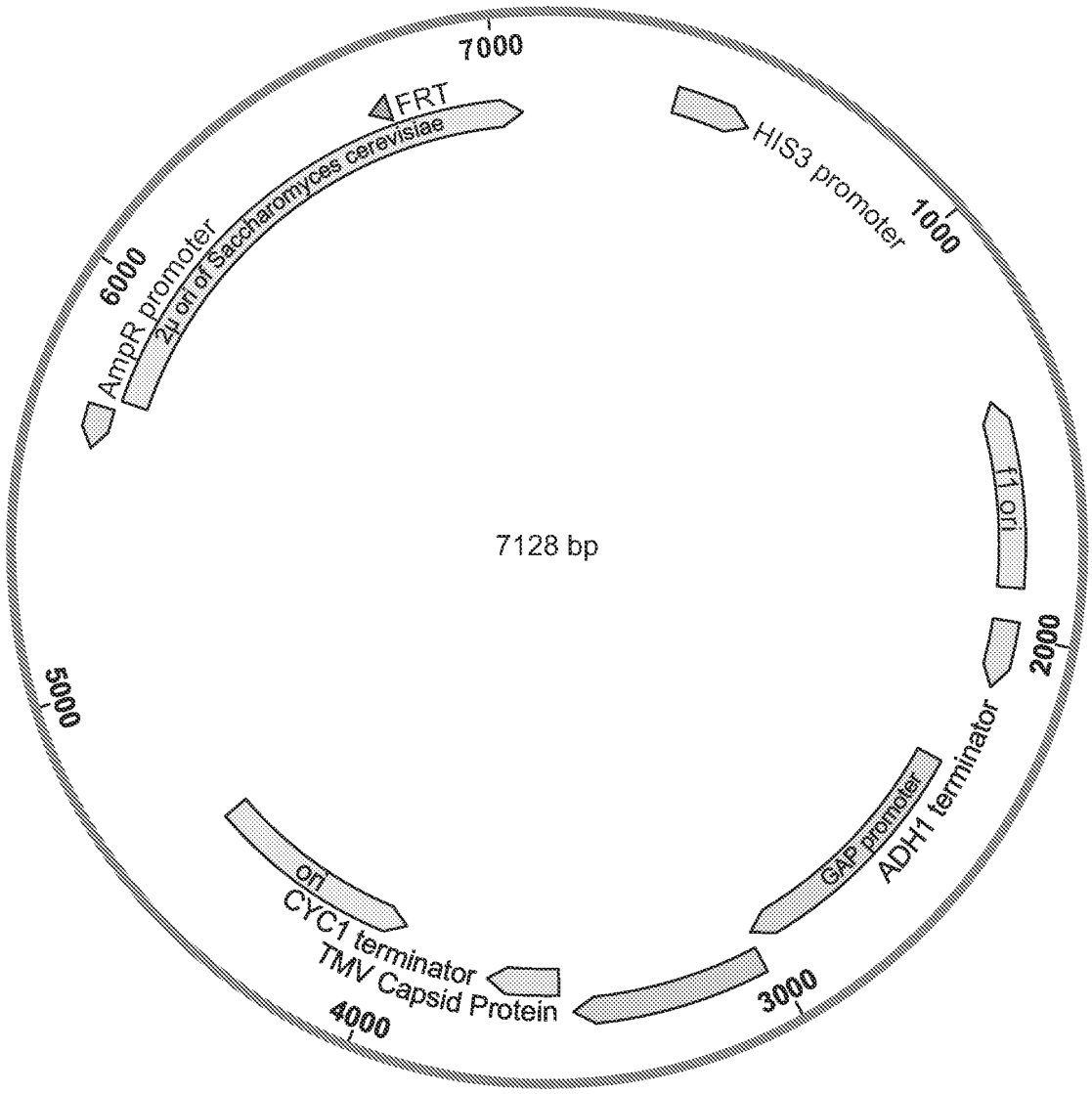

7000
FRT
HIS3 promoter
1000
6000
AmpR promoter
2µ ori of Saccharomyces cerevisiae
f1 ori
7128 bp
2000
5000
ADH1 terminator
GAP promoter
ori
CYC1 terminator
TMV Capsid Protein
3000
4000

FIG. 5

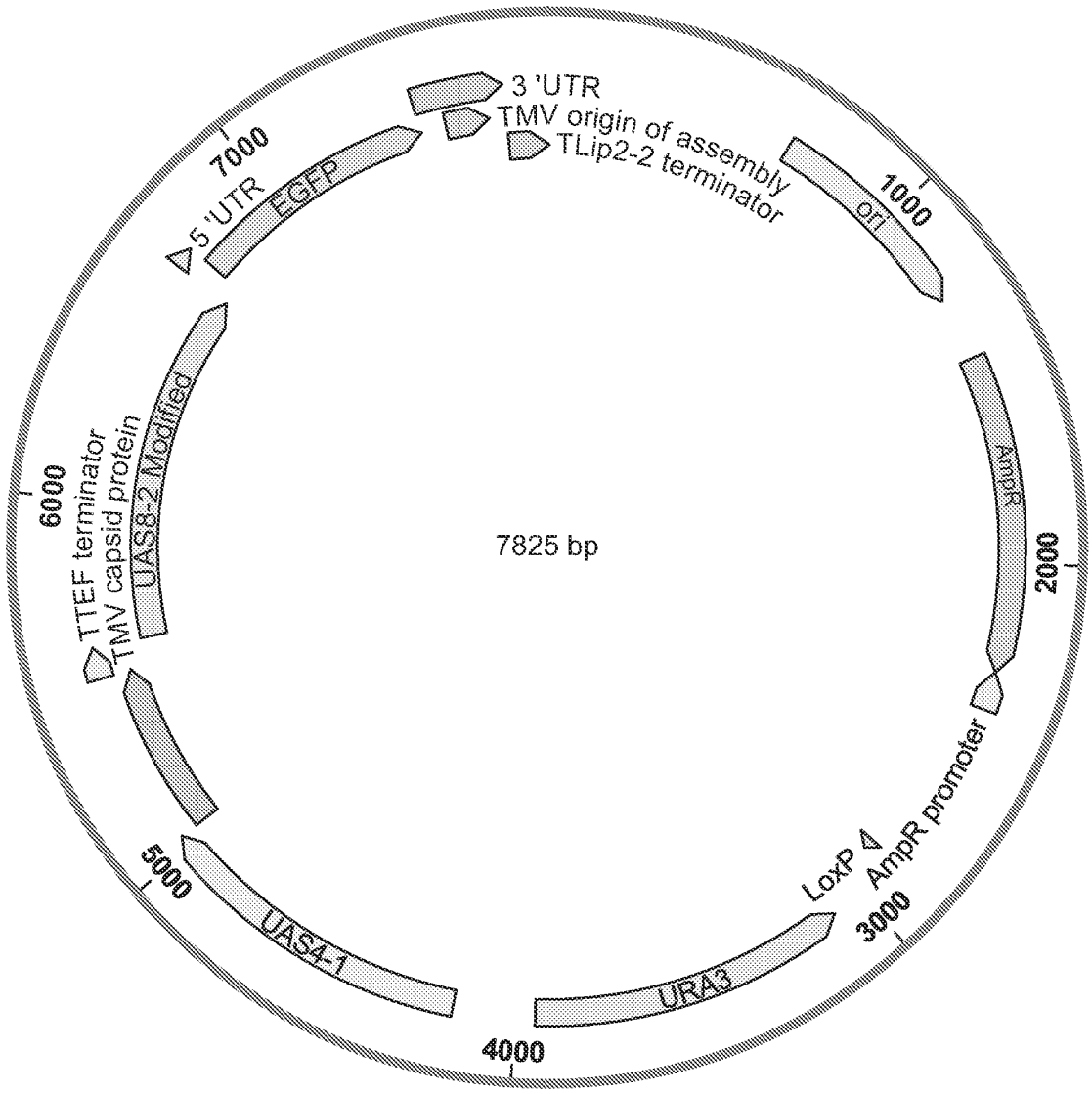

7825 bp
1000
2000
3000
4000
5000
6000
7000
3 'UTR
TMV origin of assembly
TLip2-2 terminator
ori
AmpR
AmpR promoter
LoxP
URA3
UAS4-1
TTEF terminator
TMV capsid protein
UAS8-2 Modified
5 'UTR
EGFP

FIG. 6



FIG. 7

SUBSTITUTE SHEET (RULE 26)


3' UTR4
Y1t1 retro ori
TMV origin of assembly
TMV origin of assembly
TLip2-2 terminator
Firefly Luciferase
5' UTR
7500
9003 bp
AmpR
2500
AmpR promoter
LoxP
URA3
UAS4-1 promoter
5000
TMV CP
TTEF terminator
UAS8-2 promoter

FIG. 8

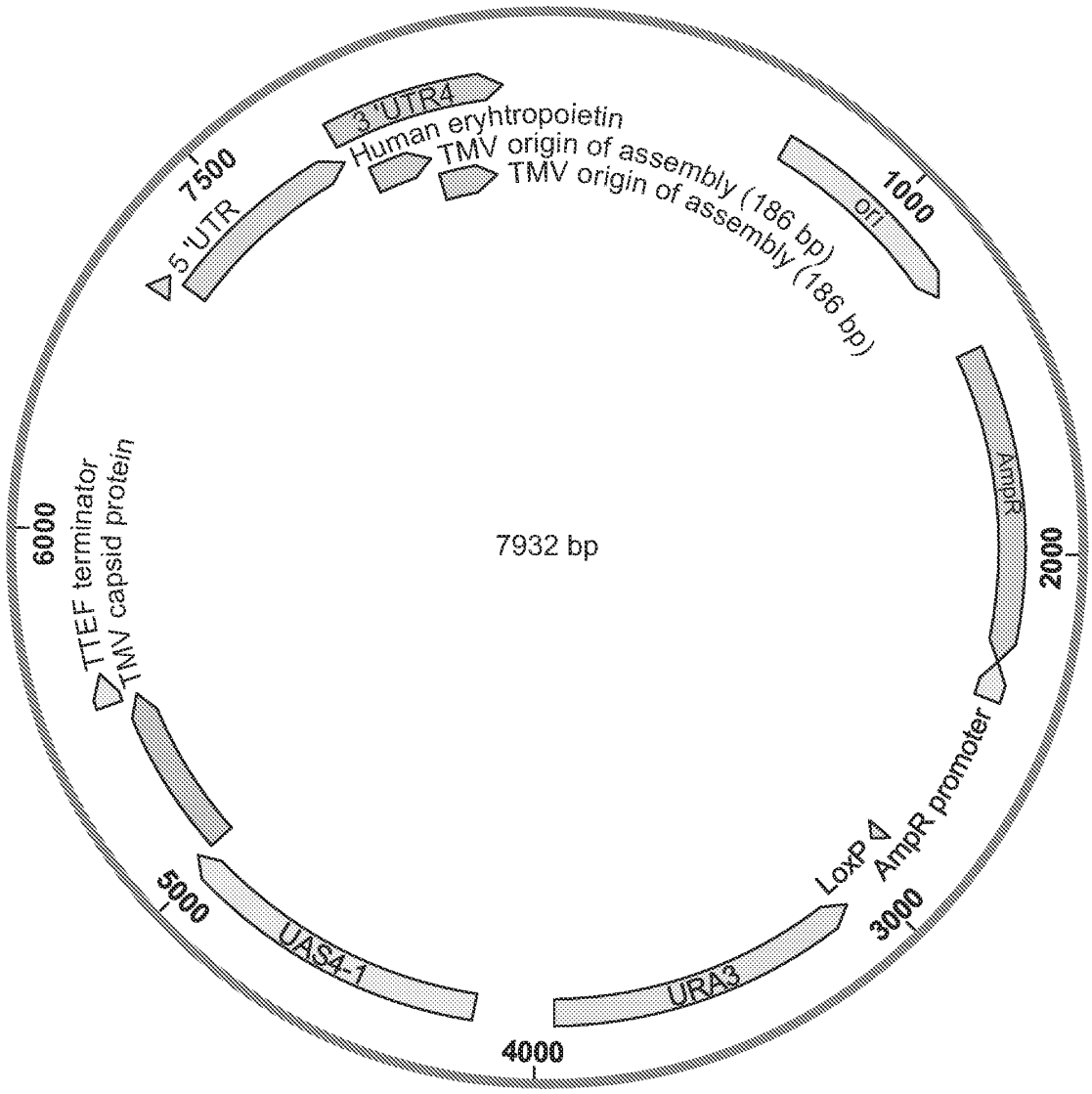

7932 bp
1000
2000
3000
4000
5000
6000
7500
3'UTR4
Human eryhtropoietin
TMV origin of assembly (186 bp)
TMV origin of assembly (186 bp)
ori
AmpR
AmpR promoter
LoxP
URA3
UAS4-1
TTEF terminator
TMV capsid protein
5'UTR

FIG. 9

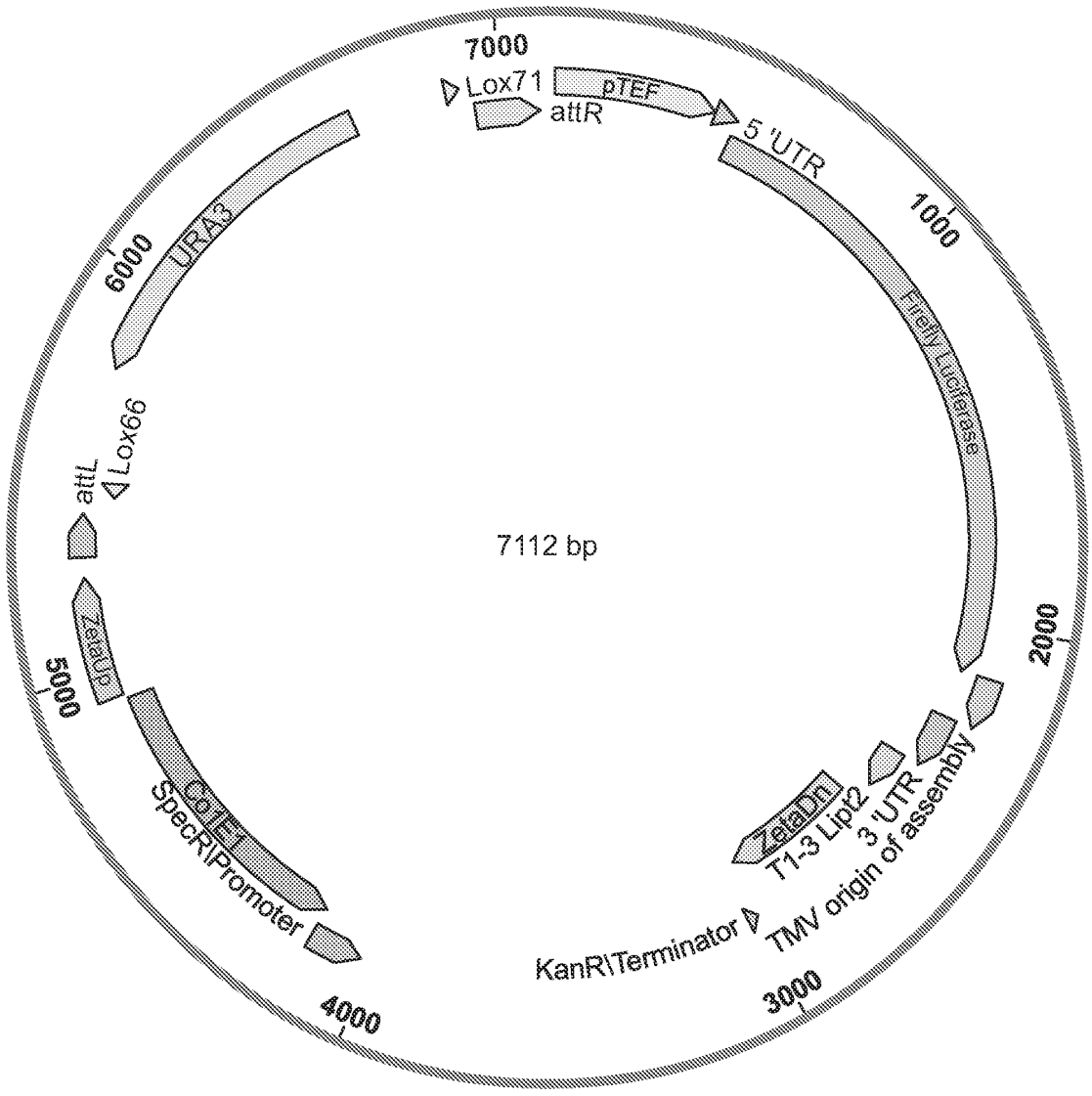

7000
Lox71
attR
pTEF
5 'UTR
1000
Firefly Luciferase
2000
3 'UTR
TMV origin of assembly
T1-3 Lip2
ZetaDn
3000
KanR\Terminator
4000
ColE1
SpecR\Promoter
5000
ZetaUp
attL
Lox66
6000
URA3
7112 bp

FIG. 10

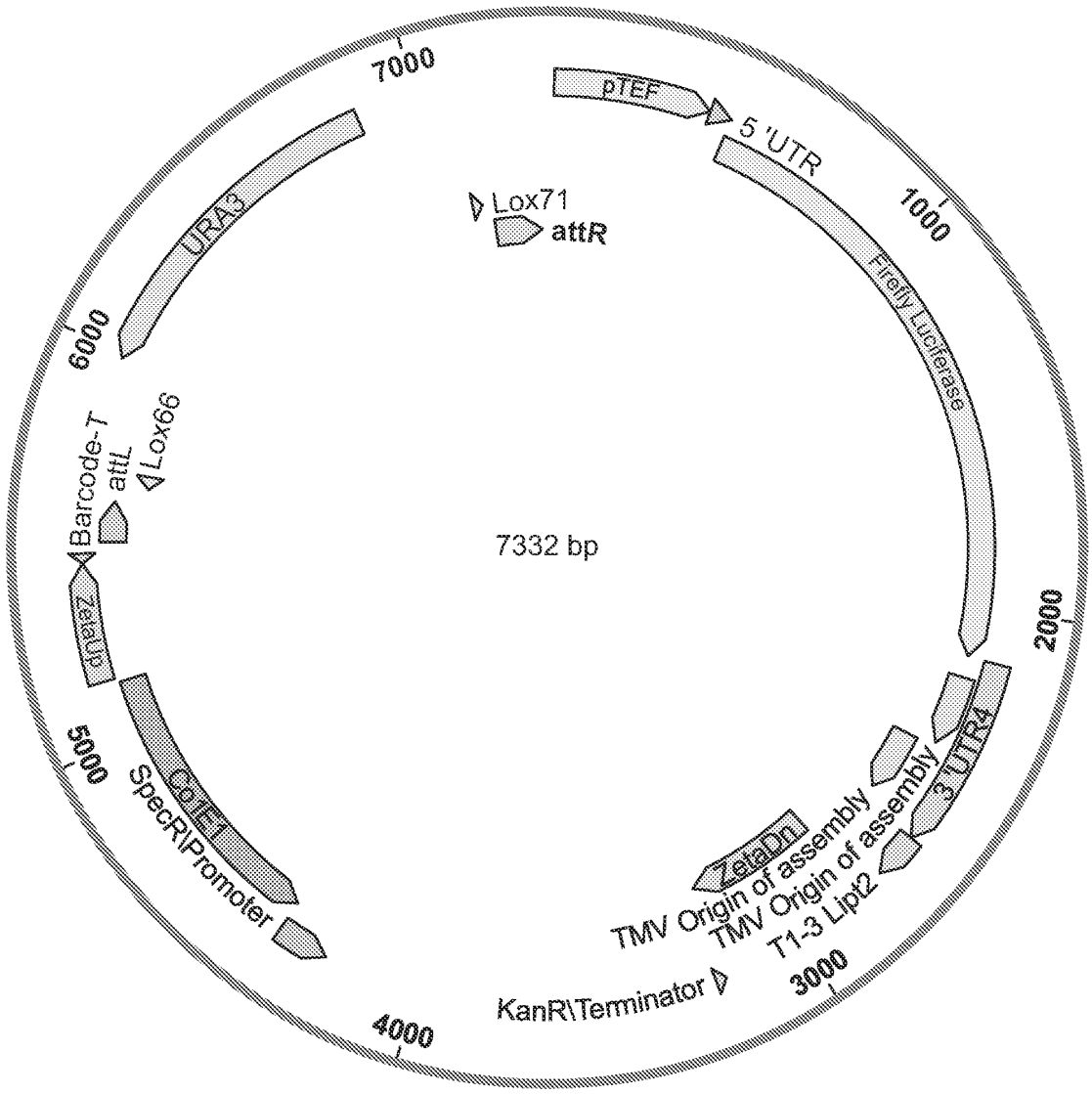

7000
pTEF
5 'UTR
1000
URA3
Lox71
attR
Firefly Luciferase
6000
Barcode-T
attL
Lox66
7332 bp
ZetaUp
2000
5000
CoTE1
SpecR\Promoter
ZetaDn
TMV Origin of assembly
TMV Origin of assembly
3 UTR4
T1-3 Lipt2
KanR\Terminator
3000
4000

FIG. 11

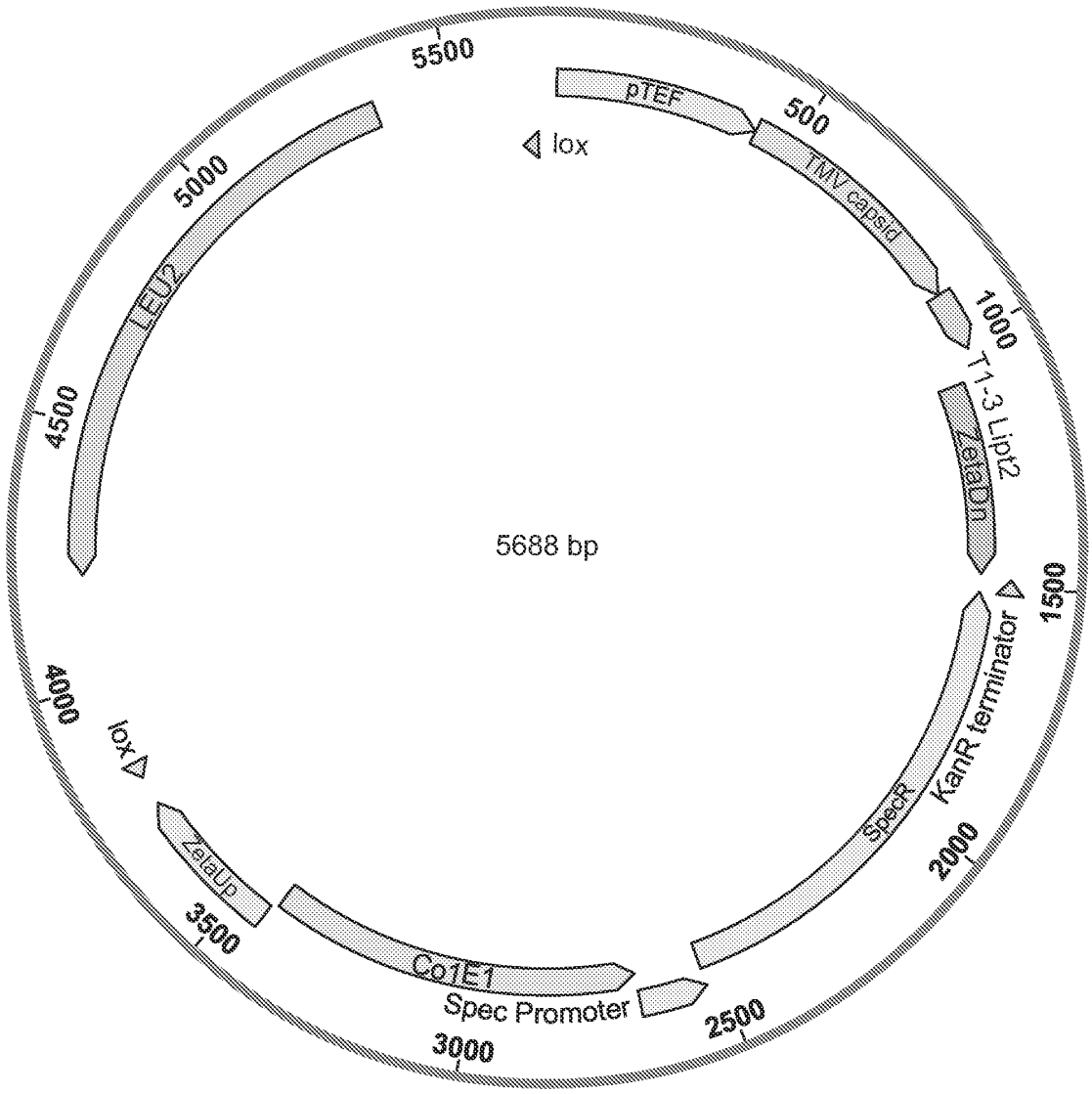

5500
pTEF
500
lox
TMV capsid
1000
T1-3 Lipt2
ZetaDn
1500
KanR terminator
2000
SpecR
2500
Spec Promoter
3000
Co1E1
3500
ZetaUp
lox
4000
4500
LEU2
5000
5688 bp

FIG. 12

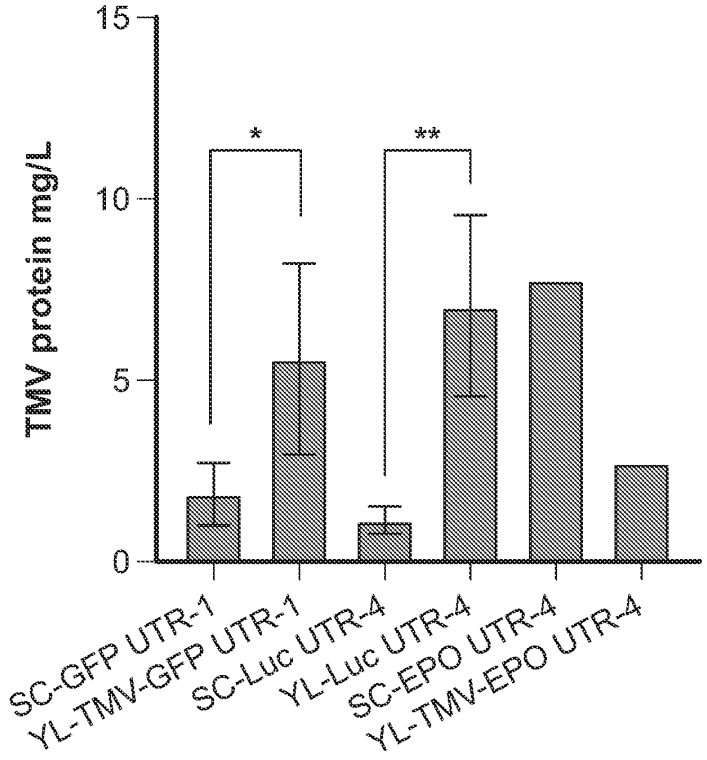


FIG. 13A

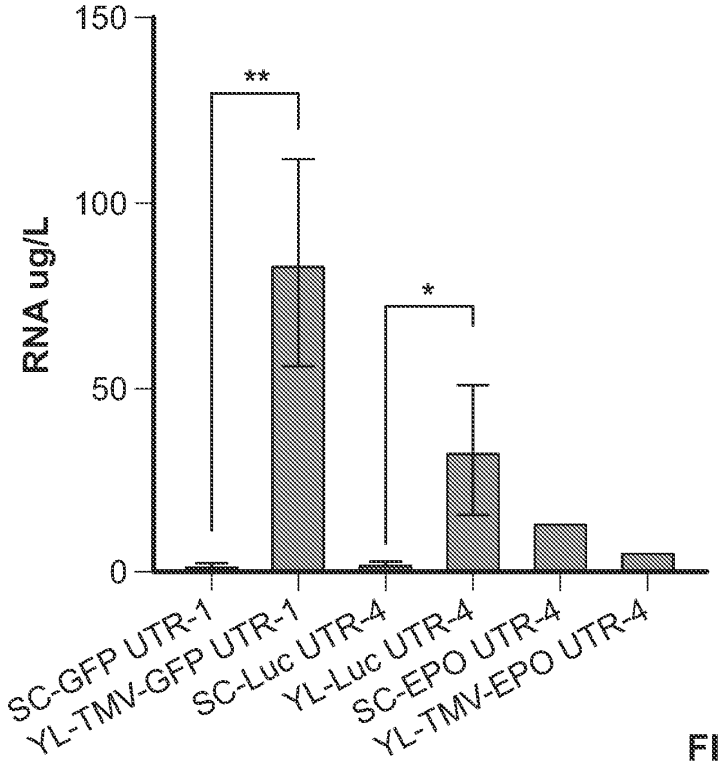


FIG. 13B

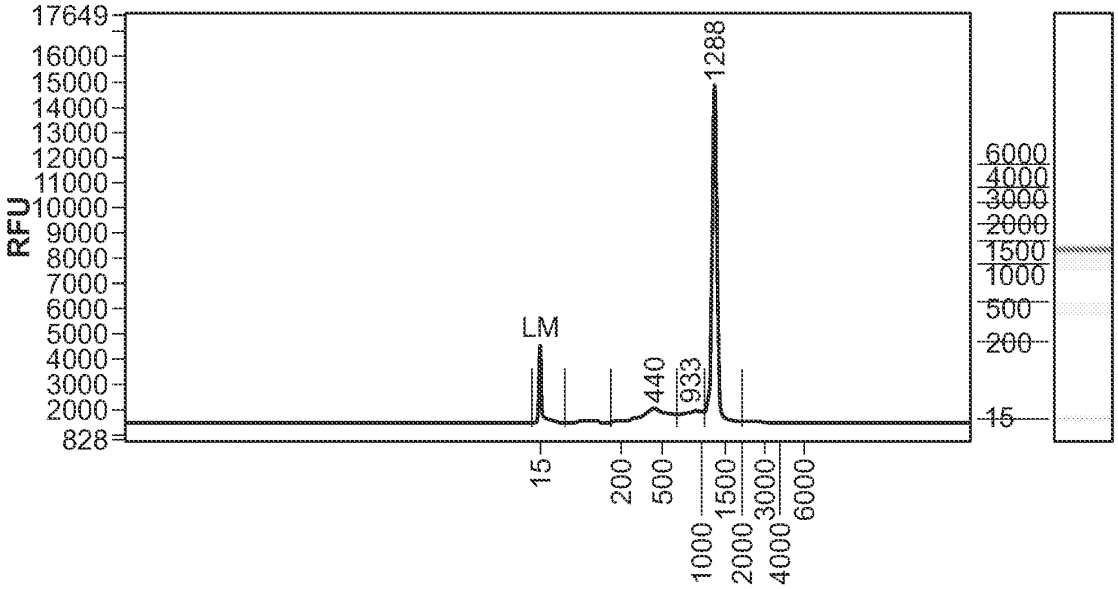


FIG. 14A

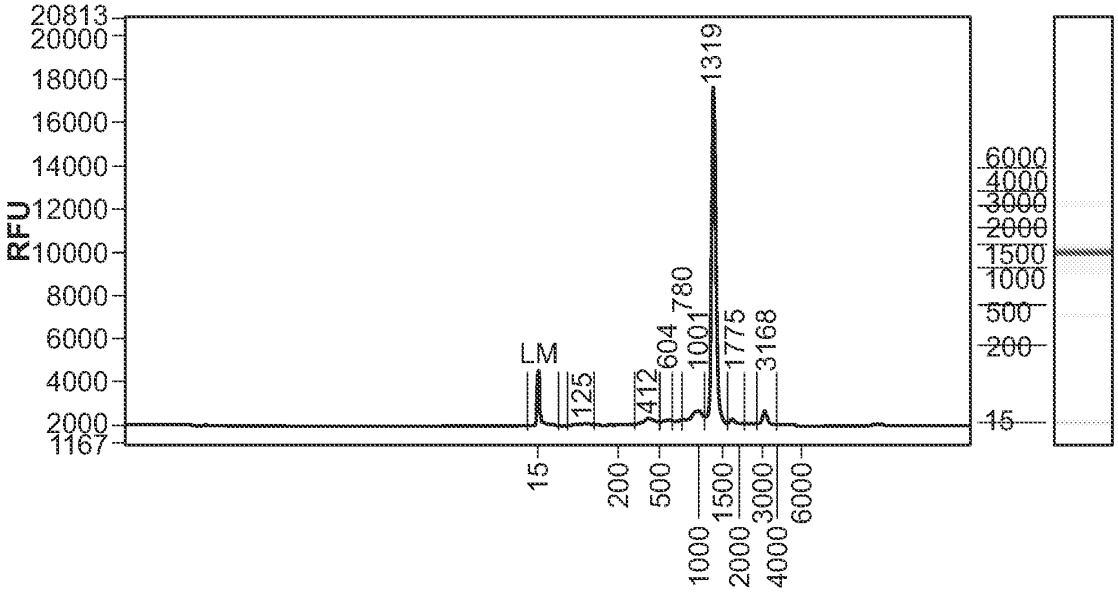


FIG. 14B

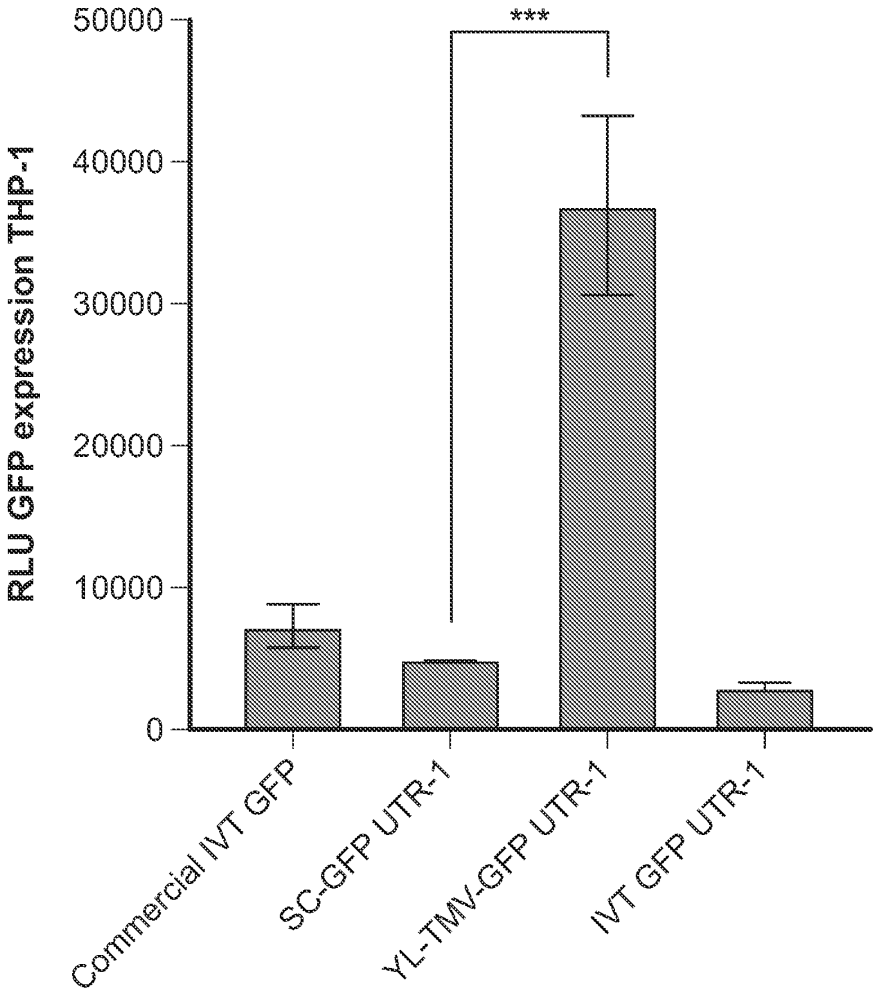

RLU GFP expression THP-1
50000
40000
30000
20000
10000
0
***
Commercial IVT GFP
SC-GFP UTR-1
YL-TMV-GFP UTR-1
IVT GFP UTR-1

FIG. 15

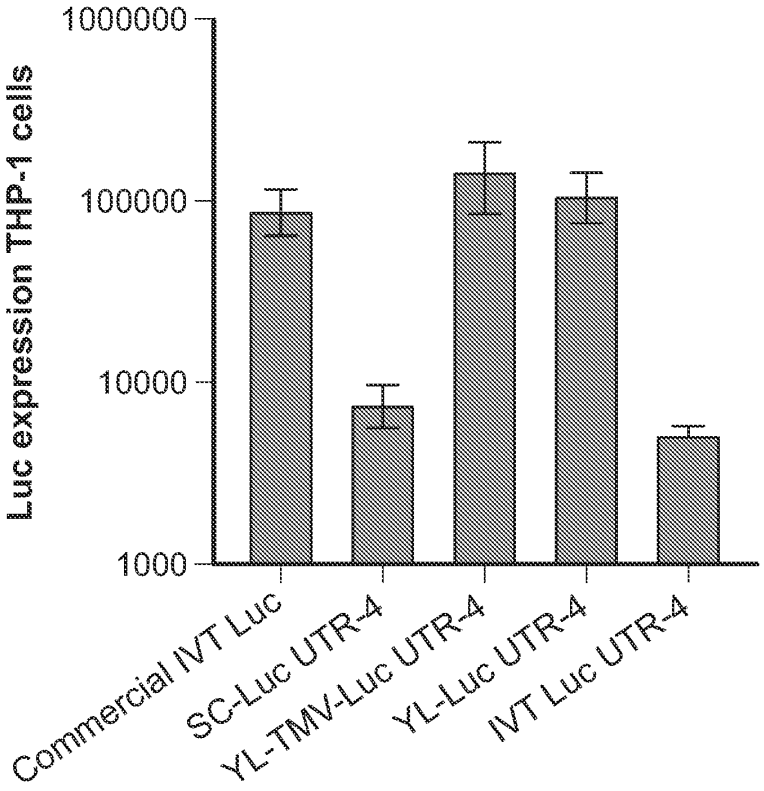


FIG. 16A

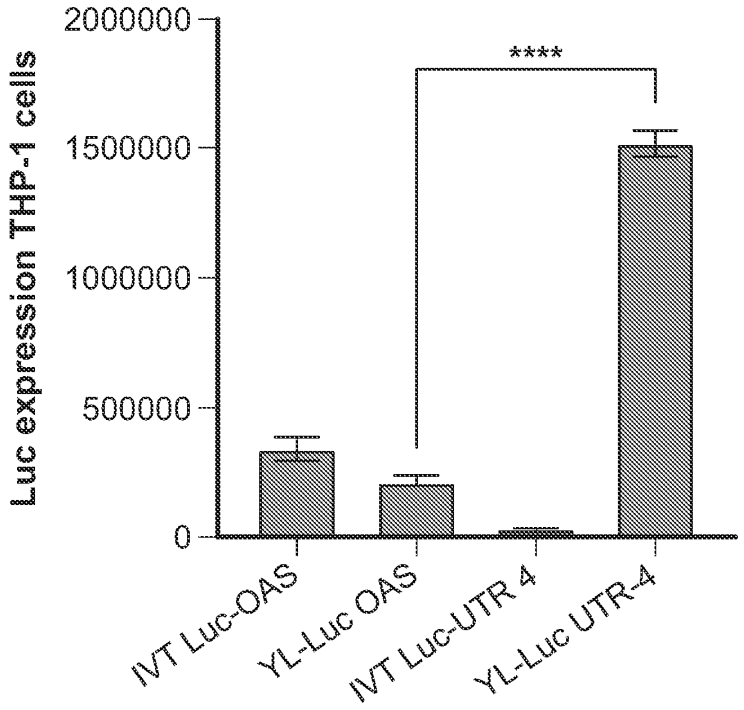


FIG. 16B

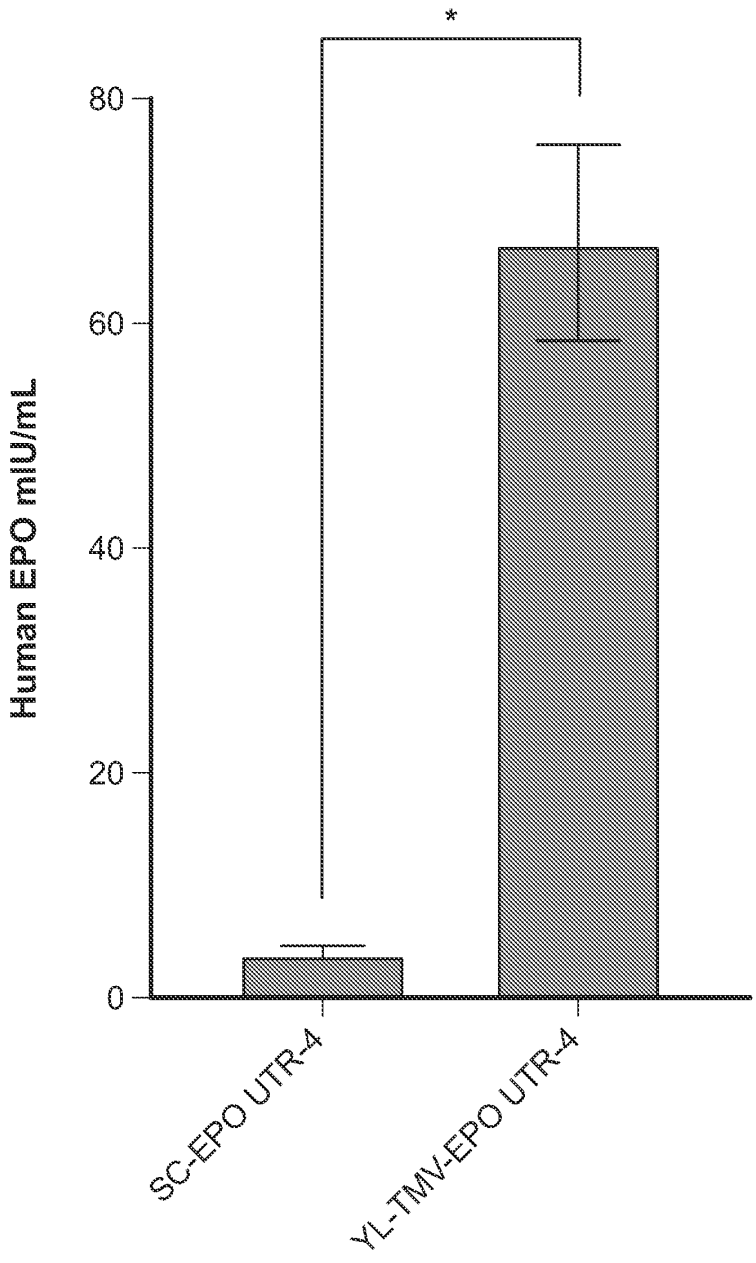


FIG. 17

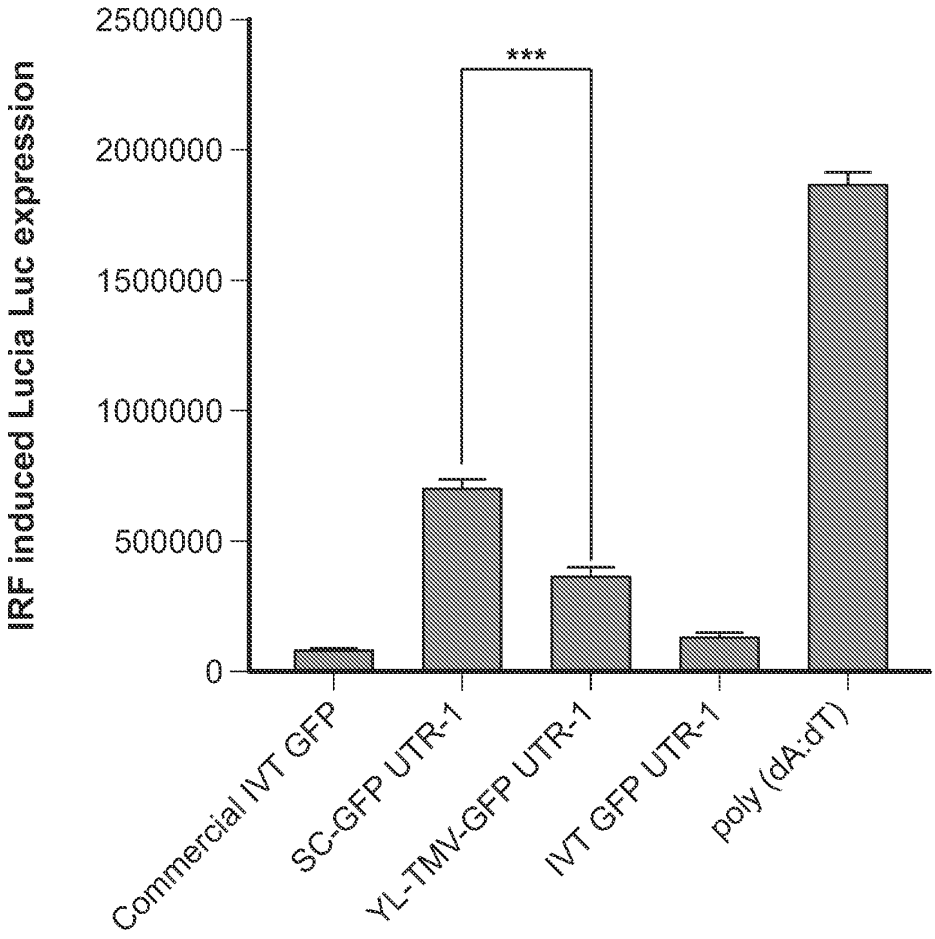

IRF induced Lucia Luc expression
2500000
2000000
1500000
1000000
500000
0
***
Commercial IVT GFP
SC-GFP UTR-1
YL-TMV-GFP UTR-1
IVT GFP UTR-1
poly (dA:dT)

FIG. 18

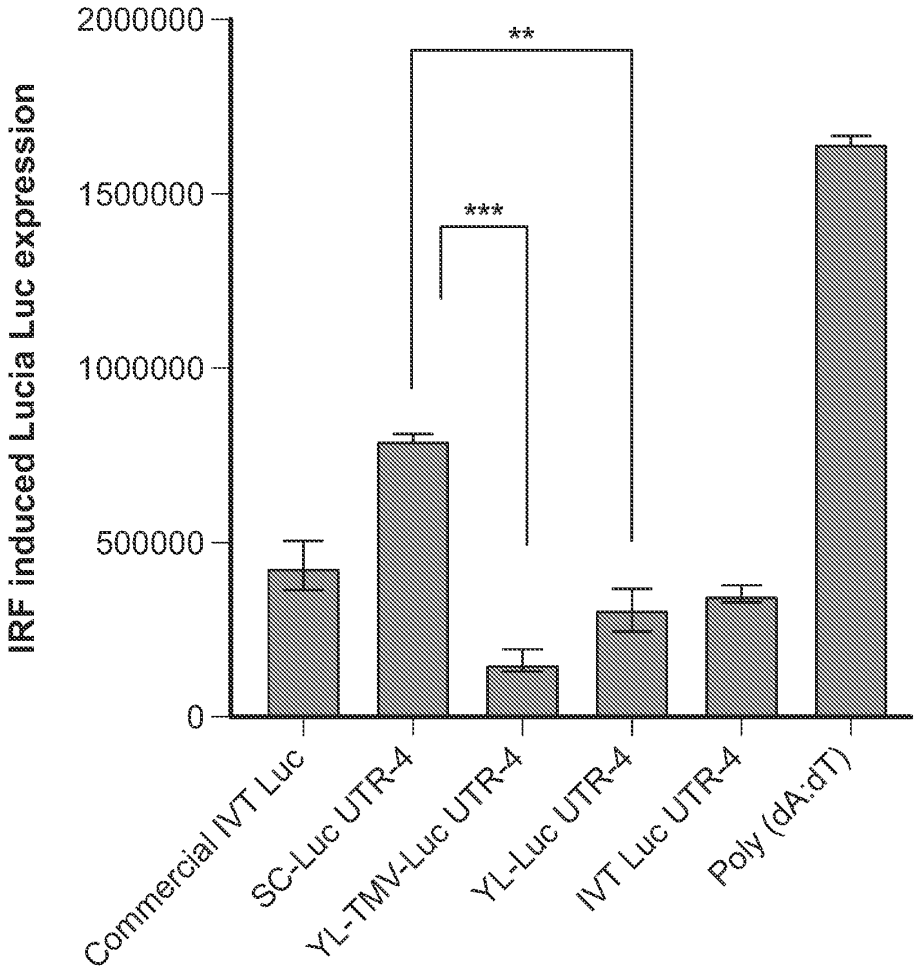

IRF induced Lucia Luc expression
2000000
1500000
1000000
500000
0
**
***
Commercial IVT Luc
SC-Luc UTR-4
YL-TMV-Luc UTR-4
YL-Luc UTR-4
IVT Luc UTR-4
Poly (dA:dT)

FIG. 19

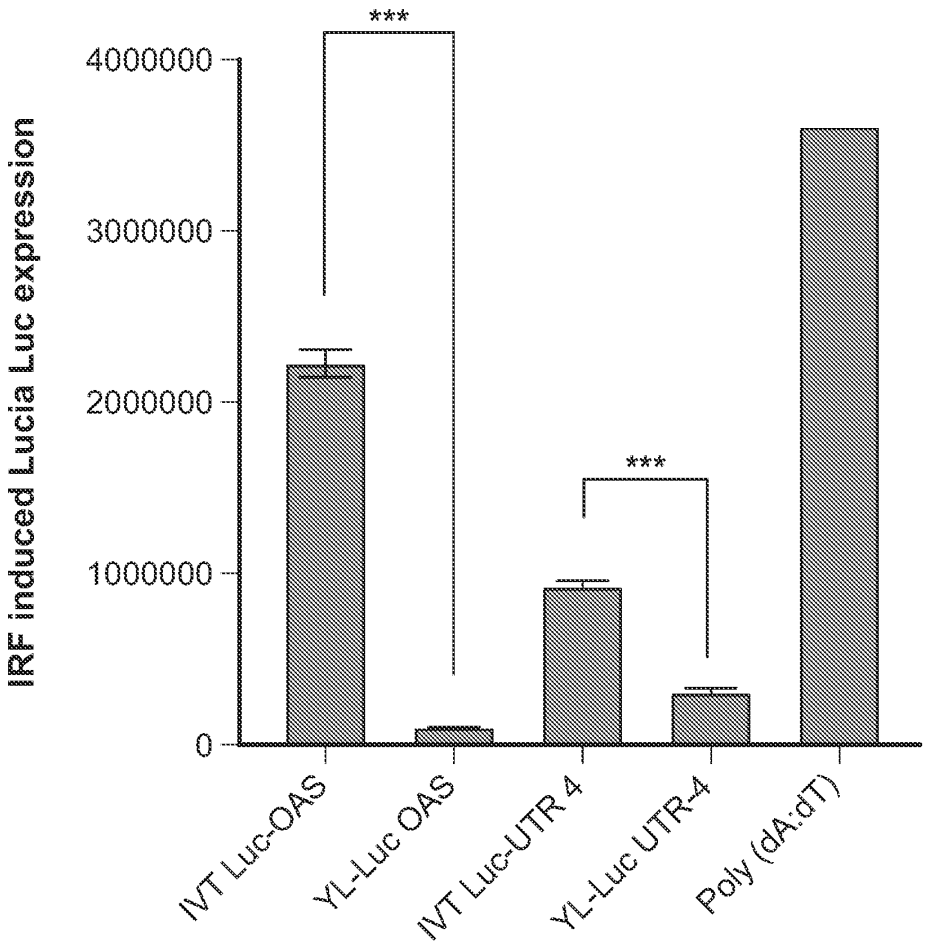

IRF induced Lucia Luc expression
4000000
3000000
2000000
1000000
0
***
***
IVT Luc-OAS
YL-Luc OAS
IVT Luc-UTR 4
YL-Luc UTR-4
Poly (dA:dT)

FIG. 20

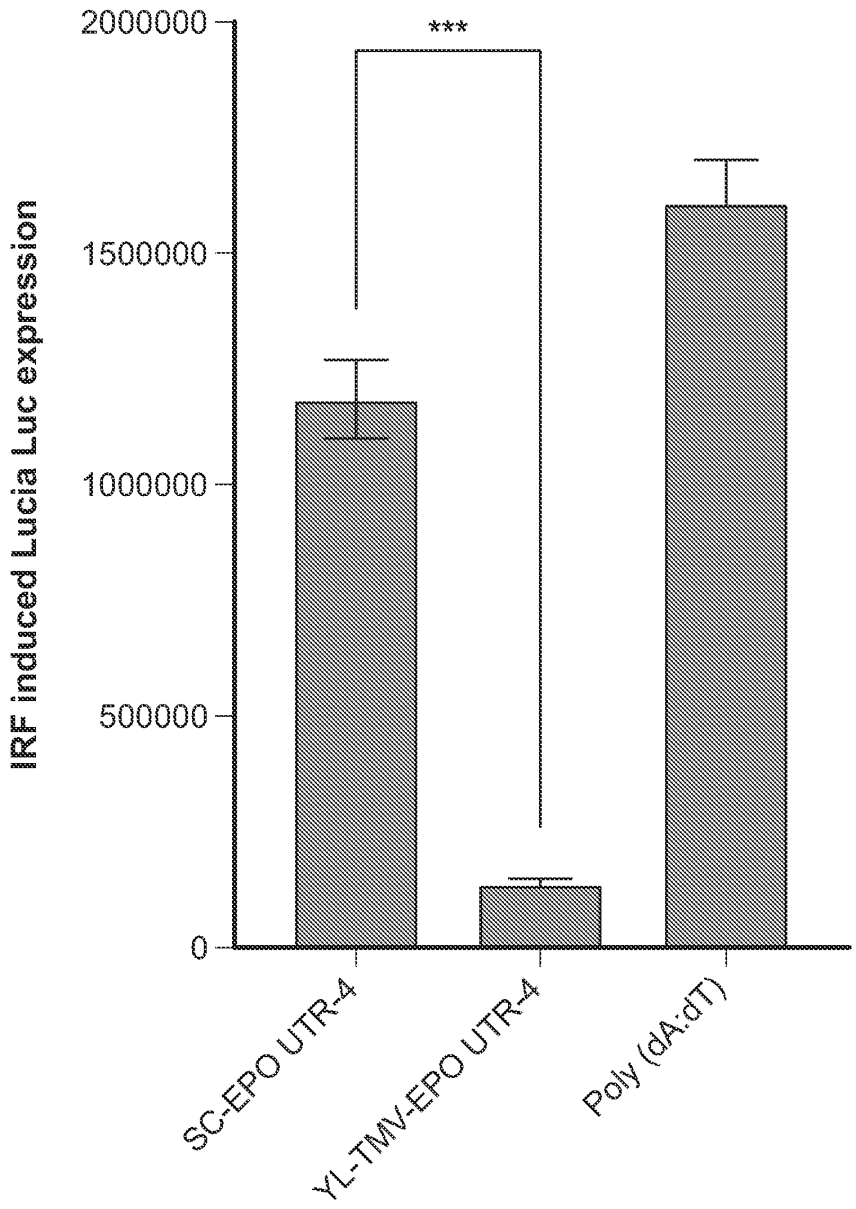

2000000
1500000
1000000
500000
0
IRF induced Lucia Luc expression
***
SC-EPO UTR-4
YL-TMV-EPO UTR-4
Poly (dA:dT)

FIG. 21

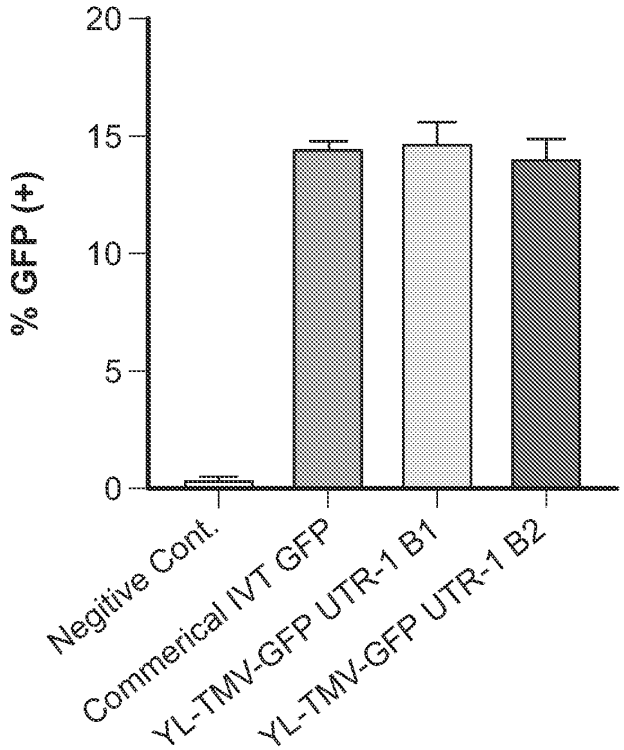


FIG. 22A

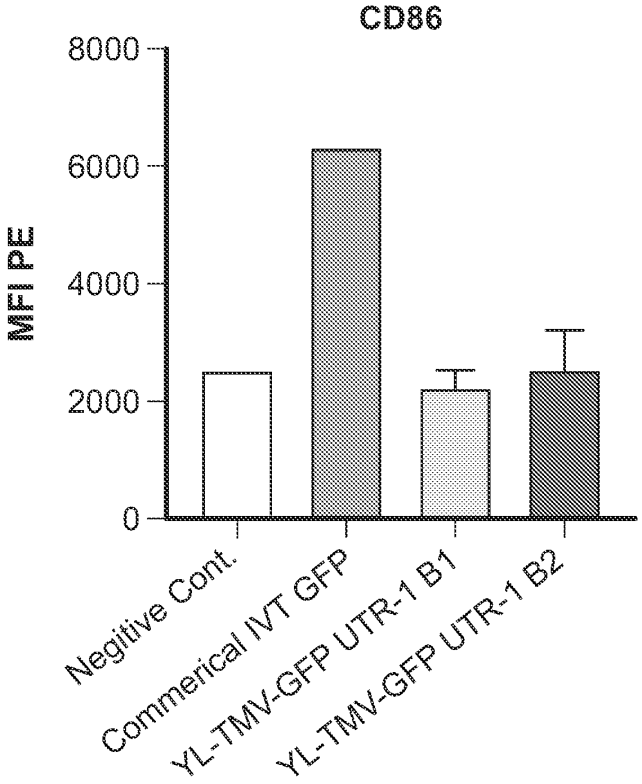


FIG. 22B

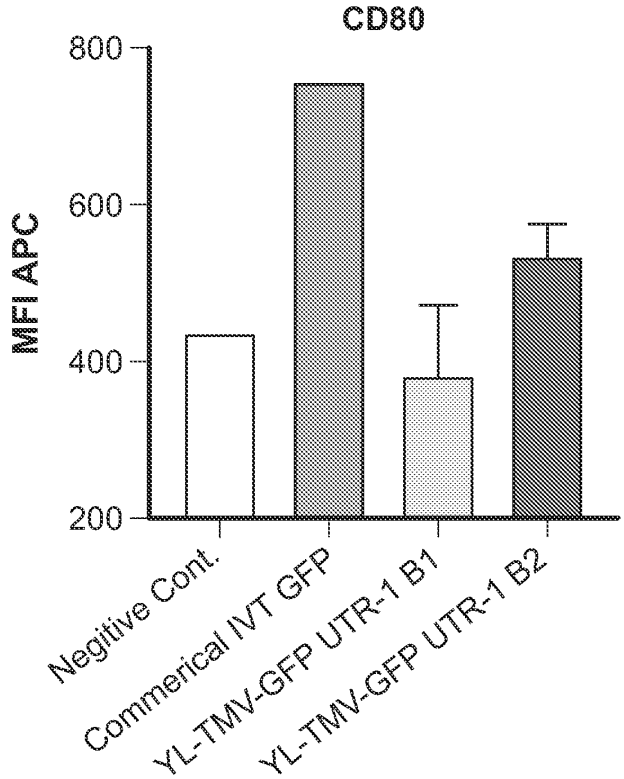


FIG. 22C

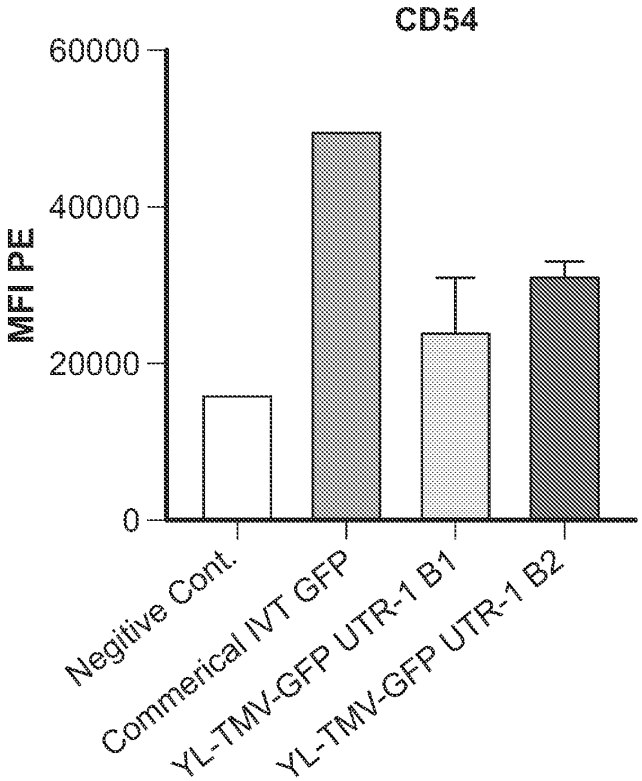


FIG. 22D

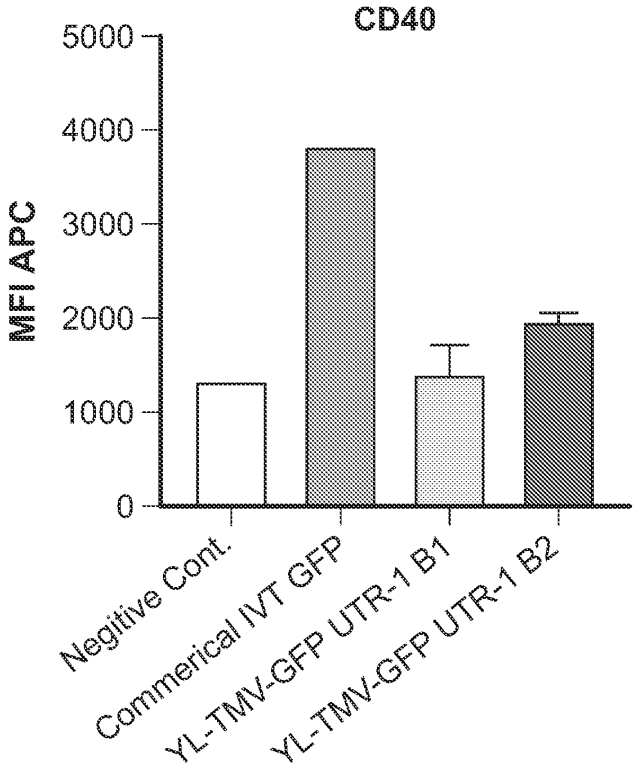


FIG. 22E

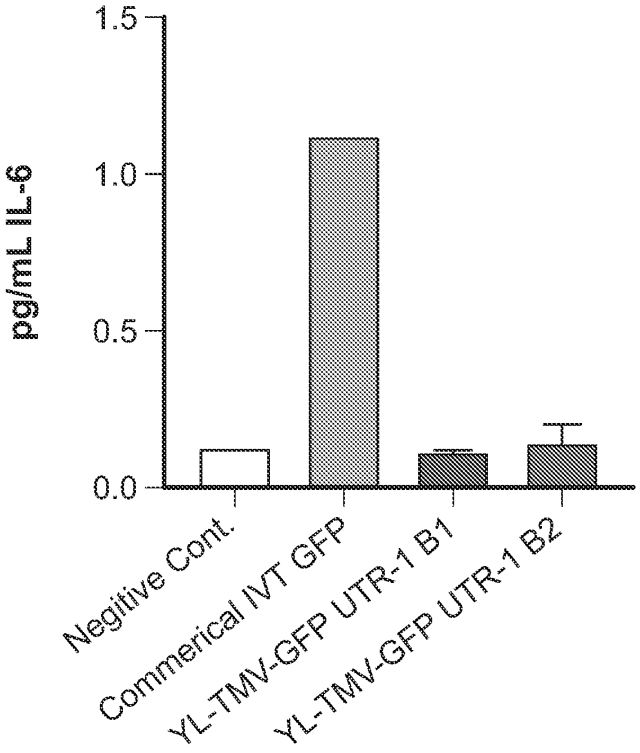


FIG. 22F

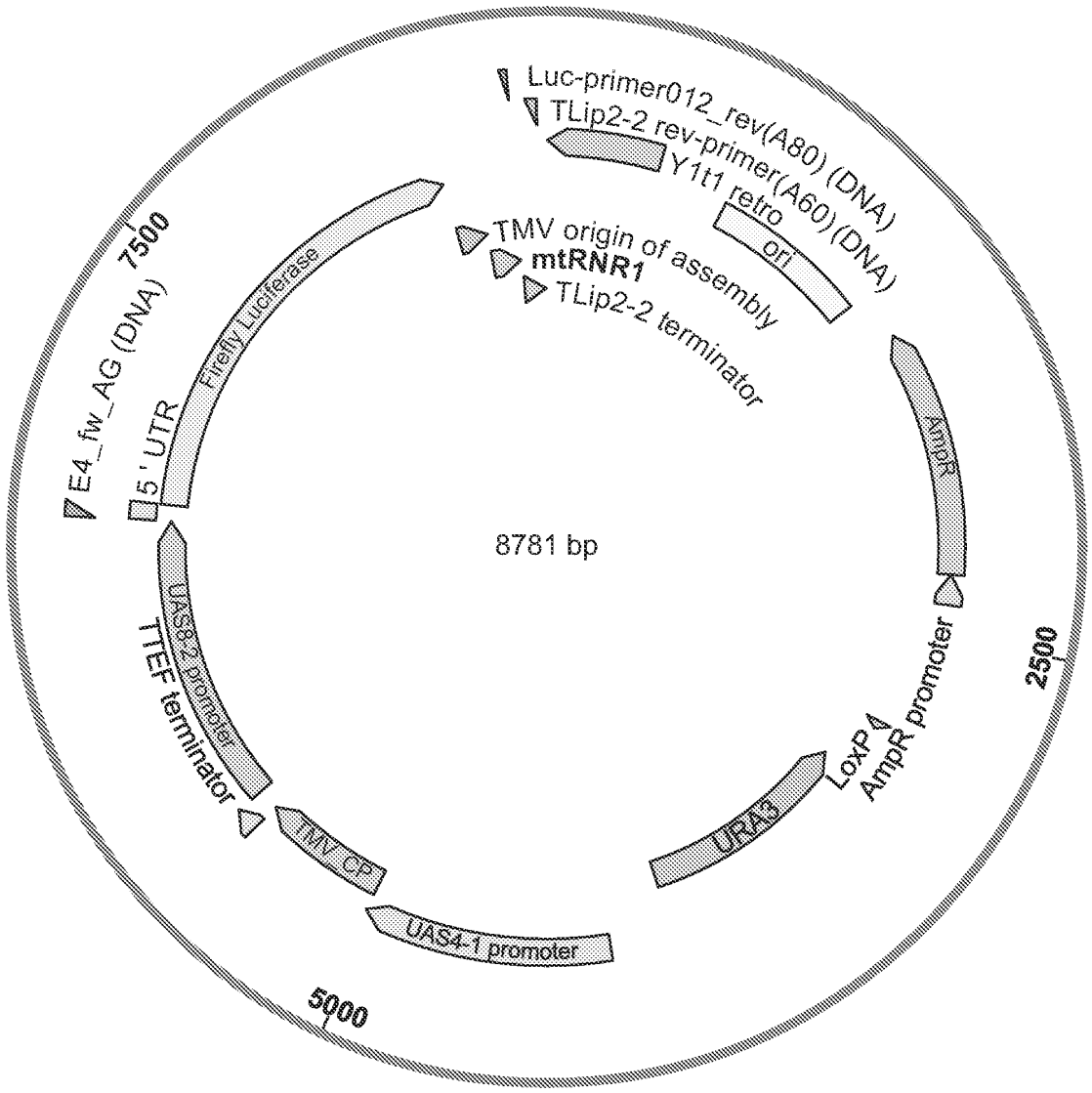

Luc-primer012_rev(A80) (DNA)
TLip2-2 rev-primer(A60) (DNA)
Y1t1 retro
ori
TMV origin of assembly
mtRNR1
TLip2-2 terminator
AmpR
AmpR promoter
LoxP
URA3
UAS4-1 promoter
TMV CP
TEF terminator
UAS8-2 promoter
Firefly Luciferase
5' UTR
E4_fw_AG (DNA)
8781 bp
2500
5000
7500

FIG. 23

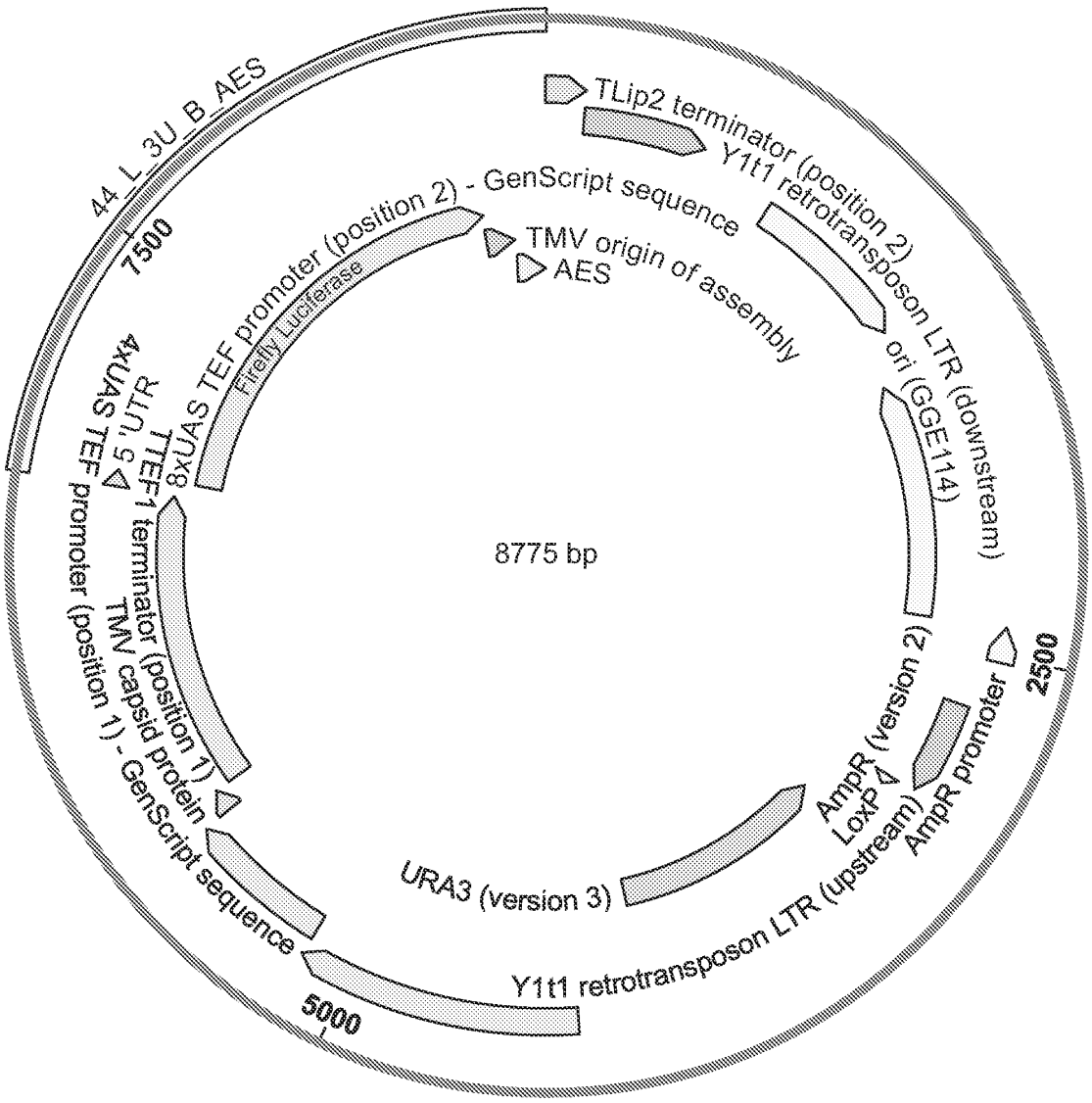

44_L_3U_B_AES
7500
TLip2 terminator (position 2)
Y1t1 retrotransposon LTR (downstream)
ori (GGE114)
AmpR promoter
2500
AmpR (version 2)
LoxP
LoxP (upstream)
URA3 (version 3)
Y1t1 retrotransposon LTR (upstream)
5000
TMV capsid protein
TEF promoter (position 1) - GenScript sequence
TEF1 terminator (position 1)
4xUAS TEF 5'UTR
8xUAS TEF promoter (position 2) - GenScript sequence
Firefly Luciferase
TMV origin of assembly
AES
8775 bp

FIG. 24

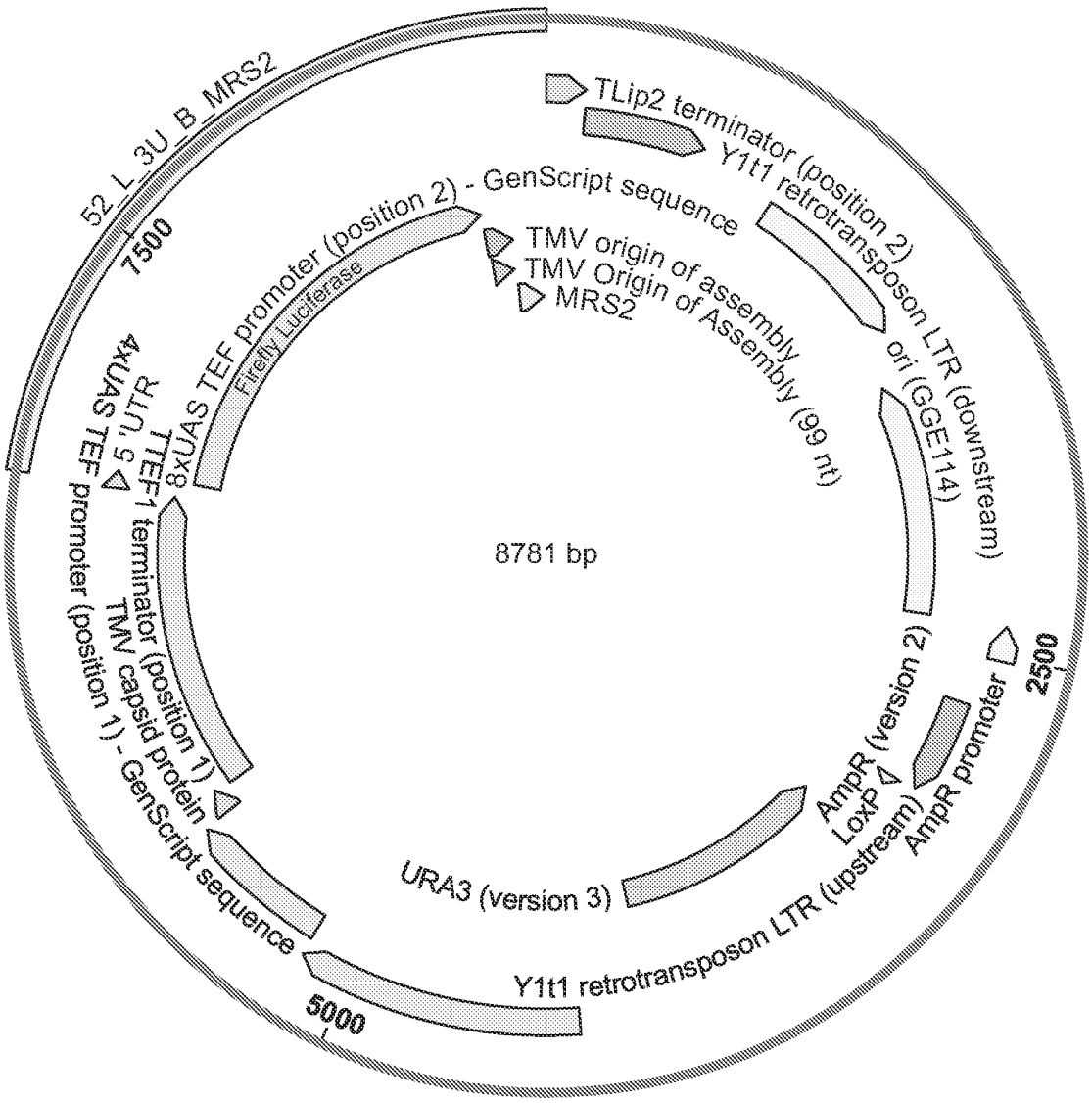

52_L_3U_B_MRS2
7500
TLip2 terminator (position 2)
Y1t1 retrotransposon LTR (downstream)
TMV origin of assembly
TMV Origin of Assembly (99 nt)
MRS2
8xUAS TEF promoter (position 2) - GenScript sequence
Firefly Luciferase
ori (GGE114)
4xUAS TEF 5'UTR
TEF1 terminator (position 1)
TMV capsid protein
8781 bp
AmpR (version 2)
LoxP
AmpR promoter
2500
TEF promoter (position 1) - GenScript sequence
URA3 (version 3)
Y1t1 retrotransposon LTR (upstream)
5000

FIG. 25

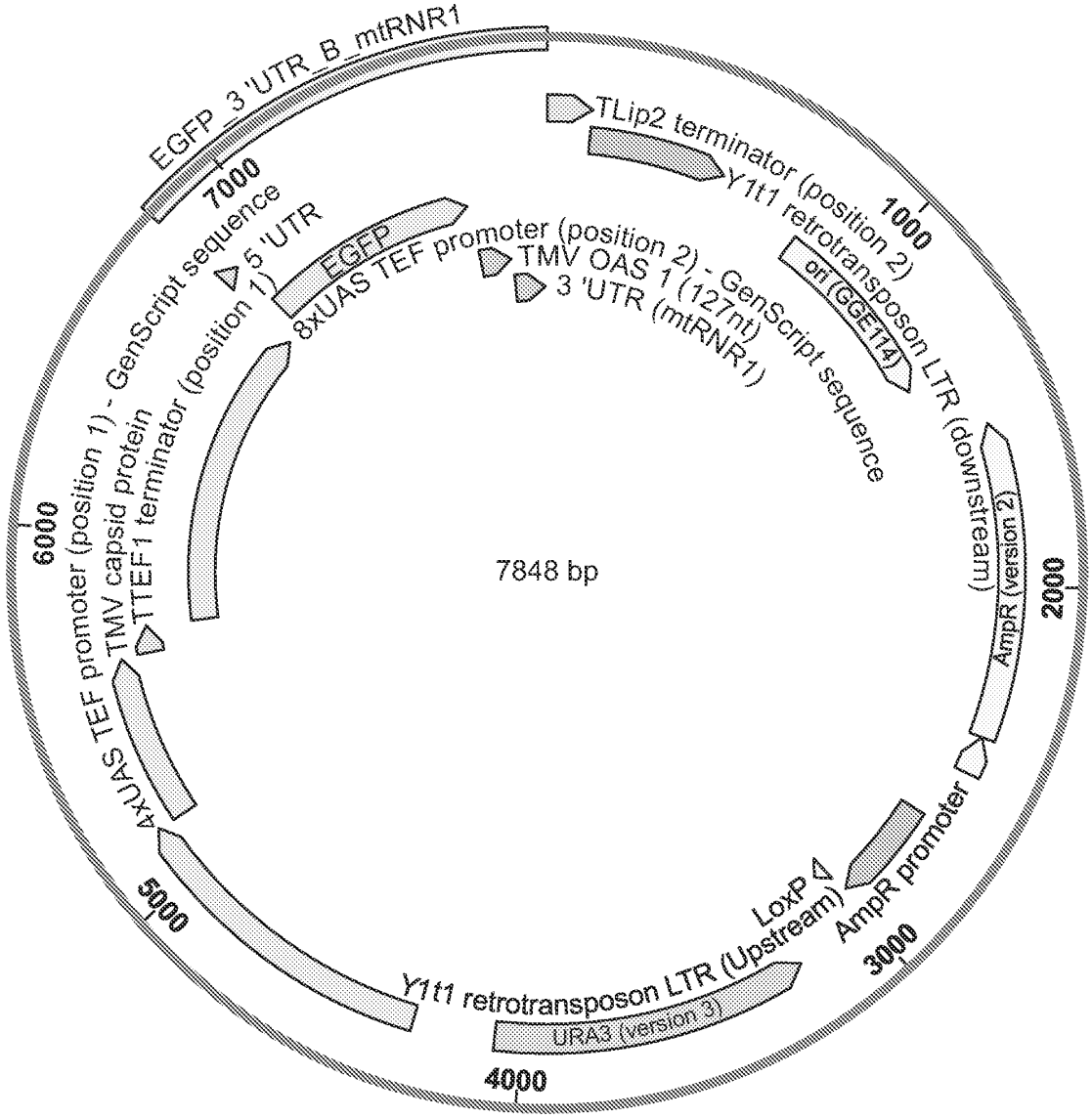

EGFP_3'UTR_B_mtRNR1
7000
5'UTR
EGFP
8xUAS TEF promoter (position 1) - GenScript sequence
TTEF1 terminator (position 1)
TMV capsid protein
4xUAS TEF promoter (position 1) - GenScript sequence
6000
5000
Y1t1 retrotransposon LTR (Upstream)
URA3 (version 3)
4000
LoxP
AmpR promoter
3000
AmpR (version 2)
2000
ori (GGE114)
Y1t1 retrotransposon LTR (downstream)
1000
TLip2 terminator (position 2)
TMV OAS 1 (127nt)
3'UTR (mtRNR1)
TEF promoter (position 2) - GenScript sequence
7848 bp

FIG. 26


EGFP_3'UTR_B_AES
7000
EGFP
5' UTR
(position 1)
8xUAS TEF promoter (position 1) - GenScript sequence
TMV capsid protein
TTEF1 terminator (position 1)
6000
4xUAS TEF promoter (position 1) - GenScript sequence
5000
Y1t1 retrotransposon LTR (upstream)
URA3 (version 3)
4000
LoxP
AmpR promoter
3000
AmpR (version 2)
2000
ori (GGE114)
Y1t1 retrotransposon LTR (downstream)
1000
TLip2 terminator (position 2)
TMV OAS 1 (127nt)
3' UTR (AES)
7842 bp

FIG. 27


EGFP_3' UTR_B_MRS2
7000
5' UTR
EGFP
8xUAS TEF promoter (position 1) - GenScript sequence
TTEF1 terminator (position 1)
TMV capsid protein
6000
4xUAS TEF promoter (position 1) - GenScript sequence
5000
Y1t1 retrotransposon LTR (upstream)
URA3 (version 3)
4000
LoxP
AmpR promoter
3000
AmpR (version 2)
2000
ori (GGE114)
Y1t1 retrotransposon LTR (downstream)
1000
TLip2 terminator (position 2)
TMV OAS 1 (127nt)
3' UTR (MRS2)
7848 bp

FIG. 28

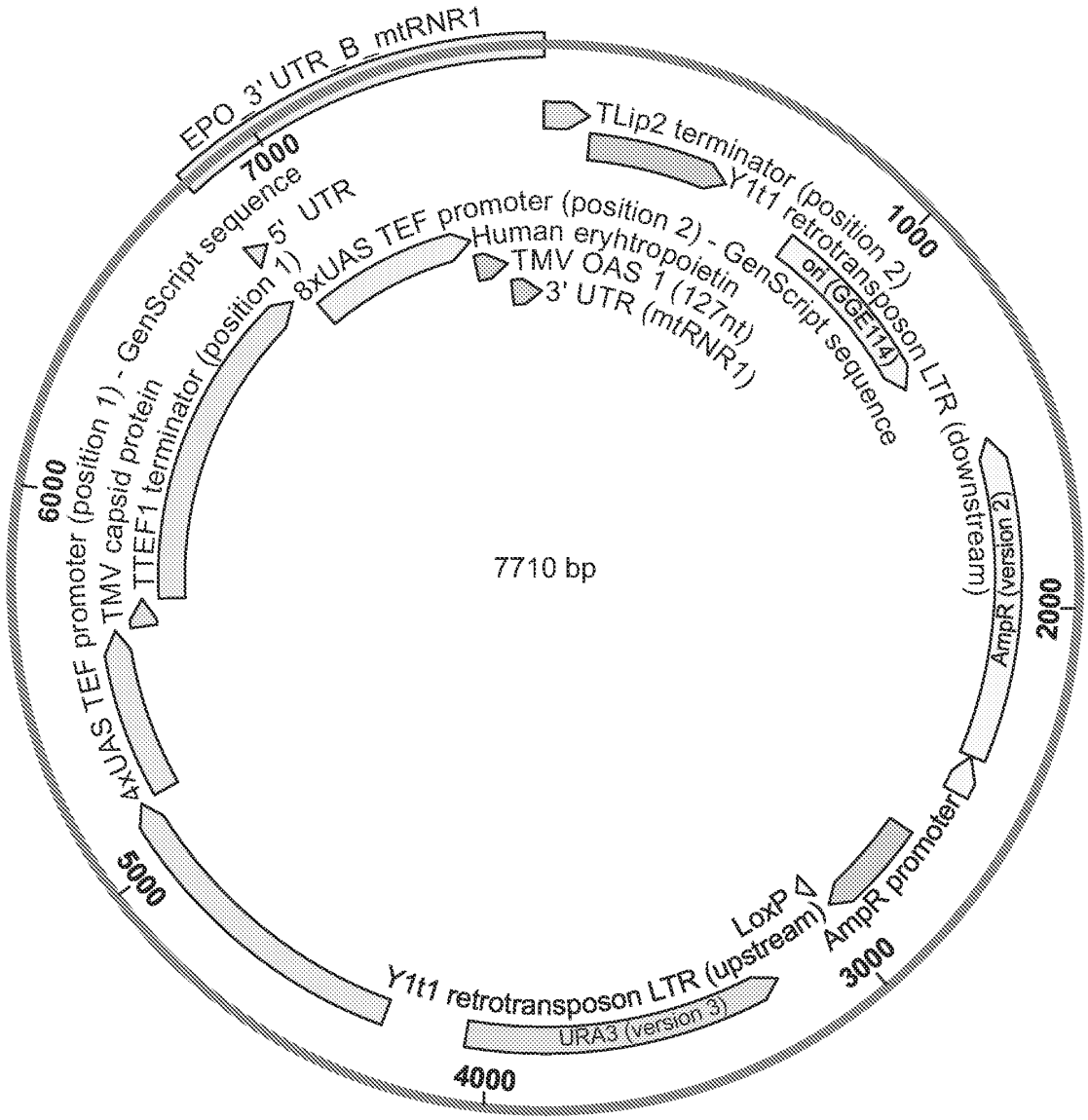

EPO_3' UTR_B_mtRNR1
7000
TLip2 terminator (position 2)
Y1t1 retrotransposon LTR (downstream)
1000
5' UTR
8xUAS TEF promoter (position 2) - GenScript sequence
Human eryhtropoietin
TMV OAS 1 (127nt)
3' UTR (mtRNR1)
ori (GGE114)
6000
TMV capsid protein
TTEF1 terminator (position 1)
7710 bp
AmpR (version 2)
2000
4xUAS TEF promoter (position 1) - GenScript sequence
5000
LoxP
AmpR promoter1
Y1t1 retrotransposon LTR (upstream)
URA3 (version 3)
3000
4000

FIG. 29

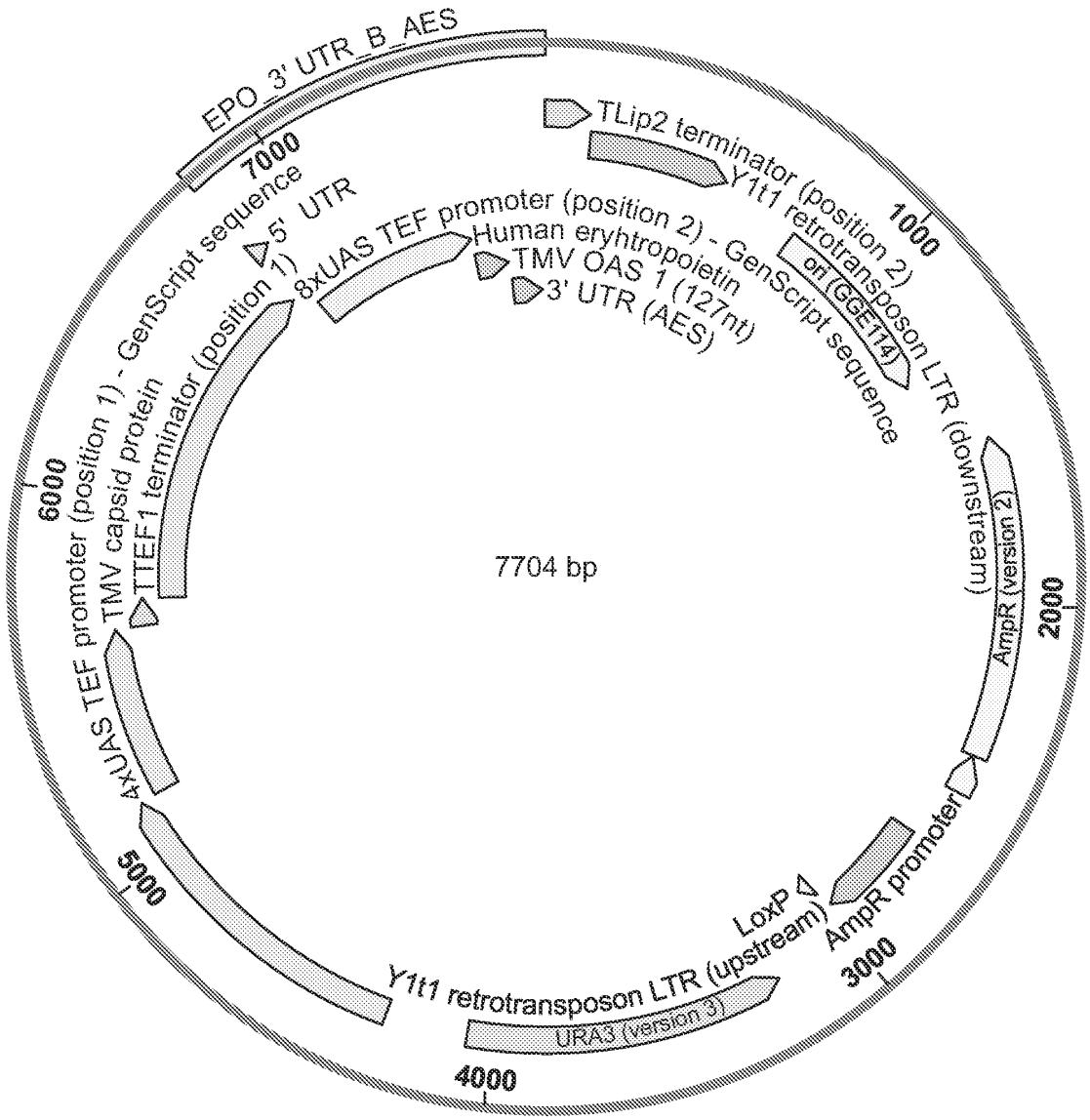

EPO_3' UTR_B_AES
7000
5' UTR
8xUAS TEF promoter (position 2) - GenScript sequence
Human eryhtropoietin
TMV OAS 1 (127nt)
3' UTR (AES)
TLip2 terminator (position 2)
Y1t1 retrotransposon LTR (downstream)
ori (GGE114)
1000
AmpR (version 2)
2000
AmpR promoter1
LoxP
3000
URA3 (version 3)
Y1t1 retrotransposon LTR (upstream)
4000
5000
4xUAS TEF promoter (position 1) - GenScript sequence
TMV capsid protein
TTEF1 terminator (position 1)
6000
7704 bp

FIG. 30

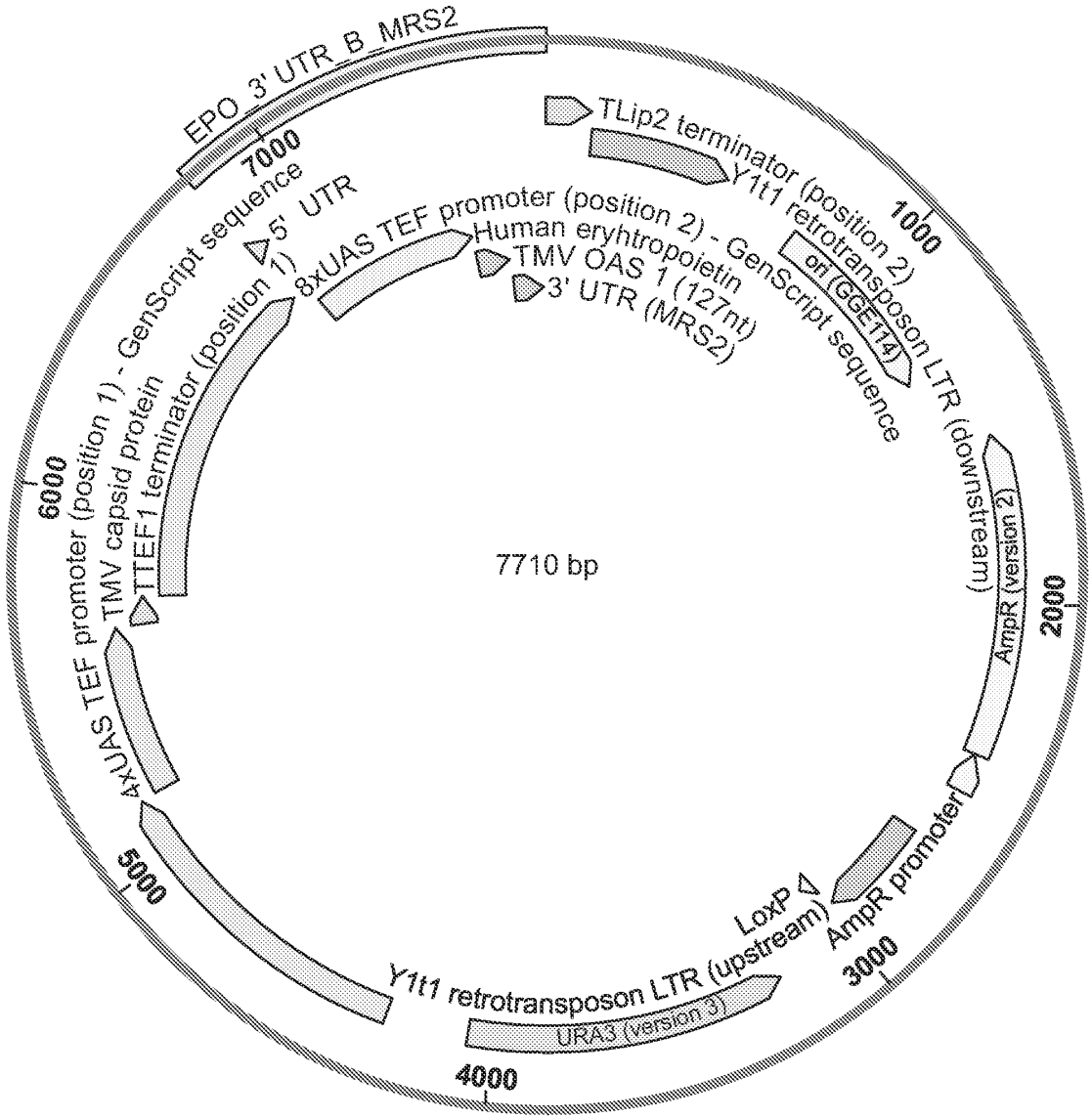

EPO 3' UTR B_MRS2
7000
TLip2 terminator (position 2)
Y1t1 retrotransposon LTR (downstream)
1000
5' UTR
1)
8xUAS TEF promoter (position 2) - GenScript sequence
Human eryhtropoietin
TMV OAS 1 (127nt)
3' UTR (MRS2)
ori (GGE114)
6000
TEF promoter (position 1) - GenScript sequence
TMV capsid protein
TEF1 terminator (position 1)
7710 bp
AmpR (version 2)
2000
4xUAS
5000
LoxP
AmpR promoter1
Y1t1 retrotransposon LTR (upstream)
URA3 (version 3)
3000
4000

FIG. 31

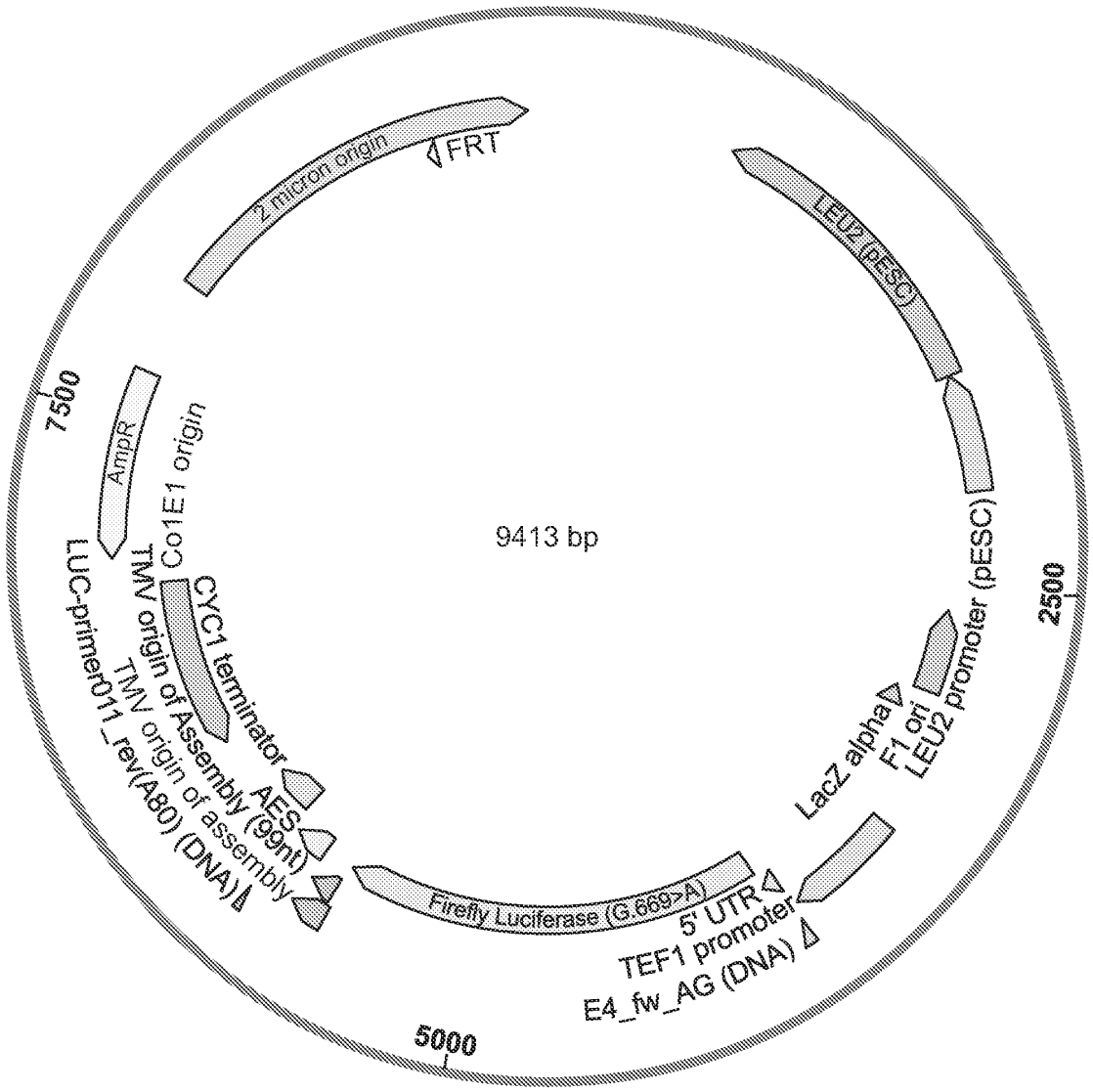

9413 bp
2 micron origin
FRT
LEU2 (pESC)
F1 ori
LEU2 promoter (pESC)
2500
LacZ alpha
5' UTR
TEF1 promoter
E4_fw_AG (DNA)
5000
Firefly Luciferase (G.669>A)
TMV origin of assembly
AES
CYC1 terminator
TMV origin of Assembly (99nt)
LUC-primer011_rev(A80) (DNA)
Co1E1 origin
AmpR
7500

FIG. 32

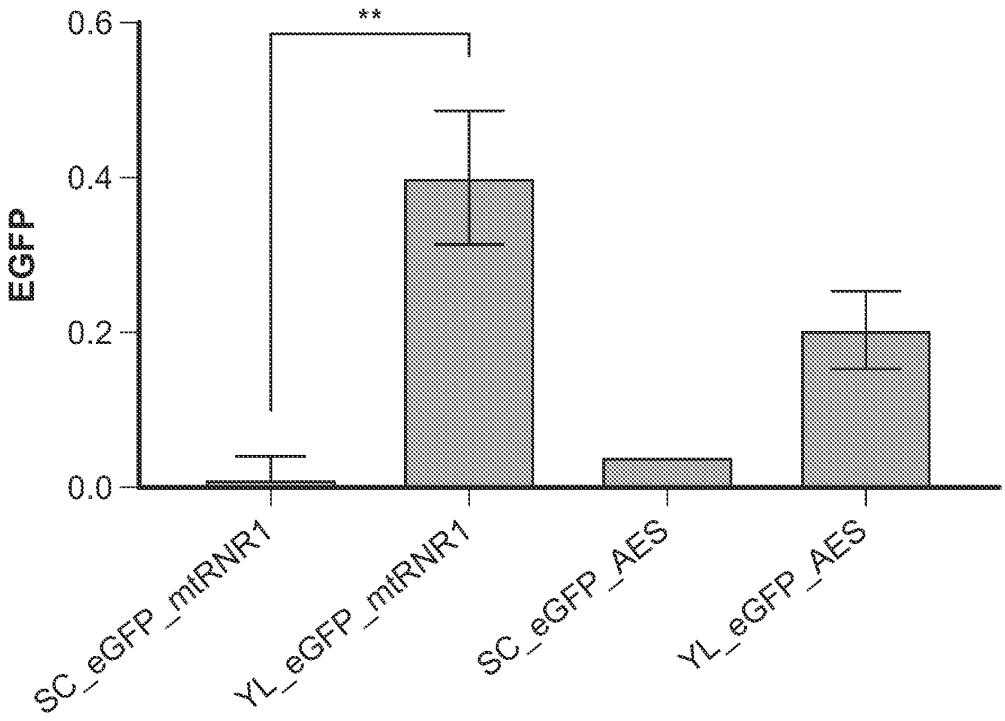

0.6
0.4
0.2
0.0
EGFP
**
SC_eGFP_mtRNR1
YL_eGFP_mtRNR1
SC_eGFP_AES
YL_eGFP_AES

FIG. 33

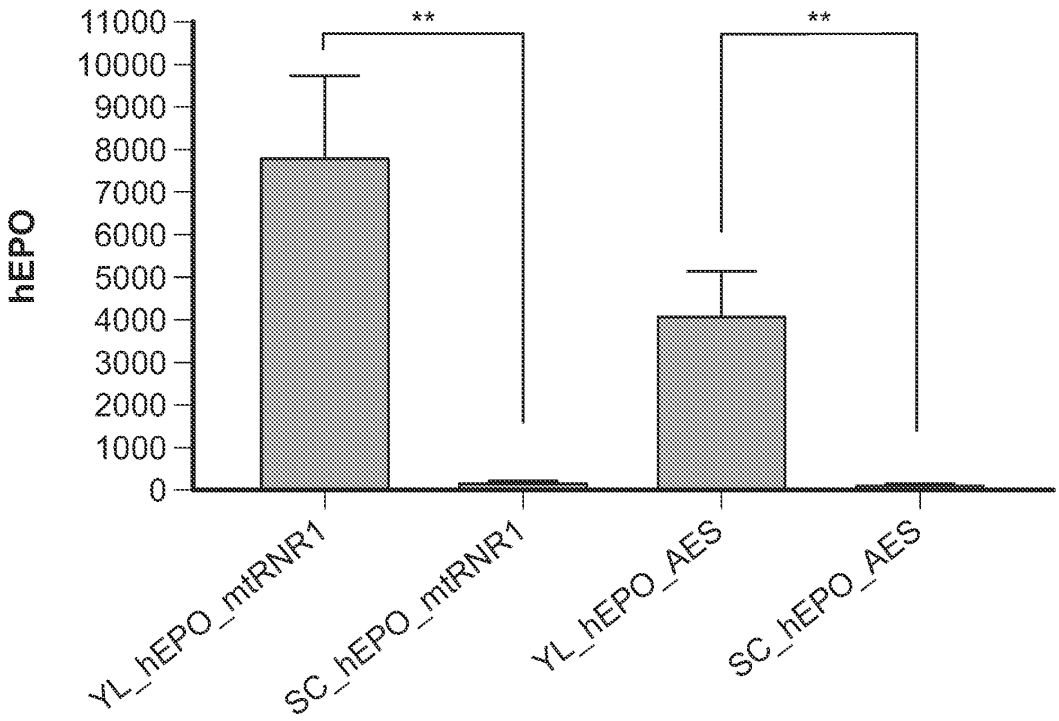

hEPO
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
**
**
YL_hEPO_mtRNR1
SC_hEPO_mtRNR1
YL_hEPO_AES
SC_hEPO_AES

FIG. 34