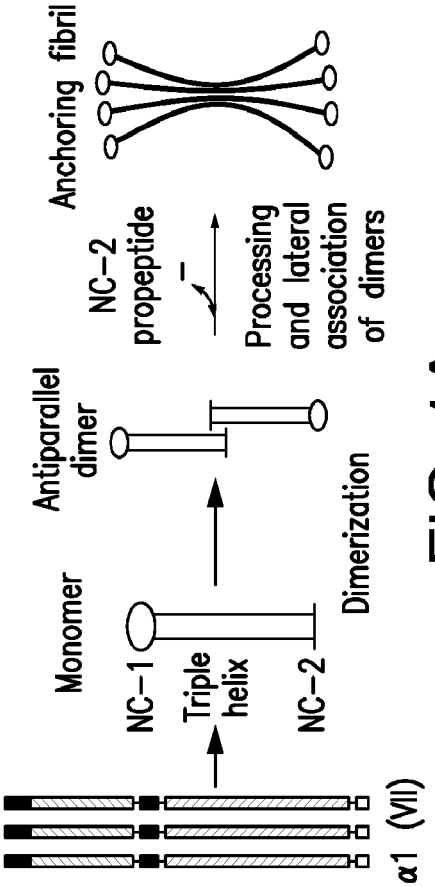

α1 (VII)
NC-1
Triple helix
NC-2
Monomer
Dimerization
Antiparallel dimer
NC-2 propeptide
Processing and lateral association of dimers
Anchoring fibril

FIG. 1A

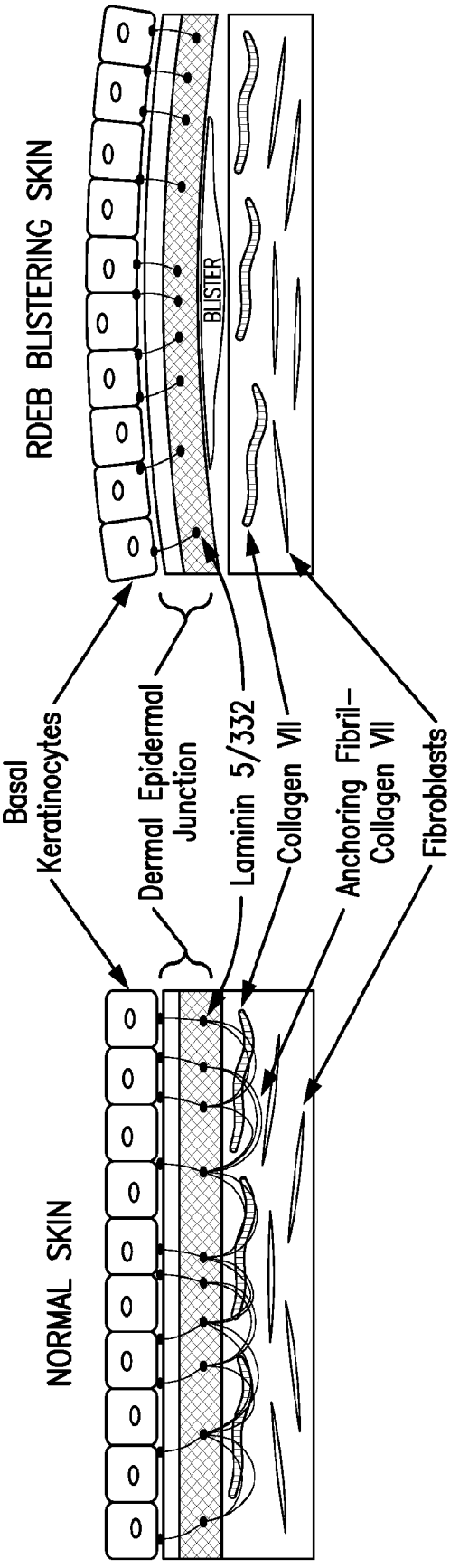

NORMAL SKIN
RDEB BLISTERING SKIN
BLISTER
Basal Keratinocytes
Dermal Epidermal Junction
Laminin 5/332
Collagen VII
Anchoring Fibril–Collagen VII
Fibroblasts

FIG. 1B

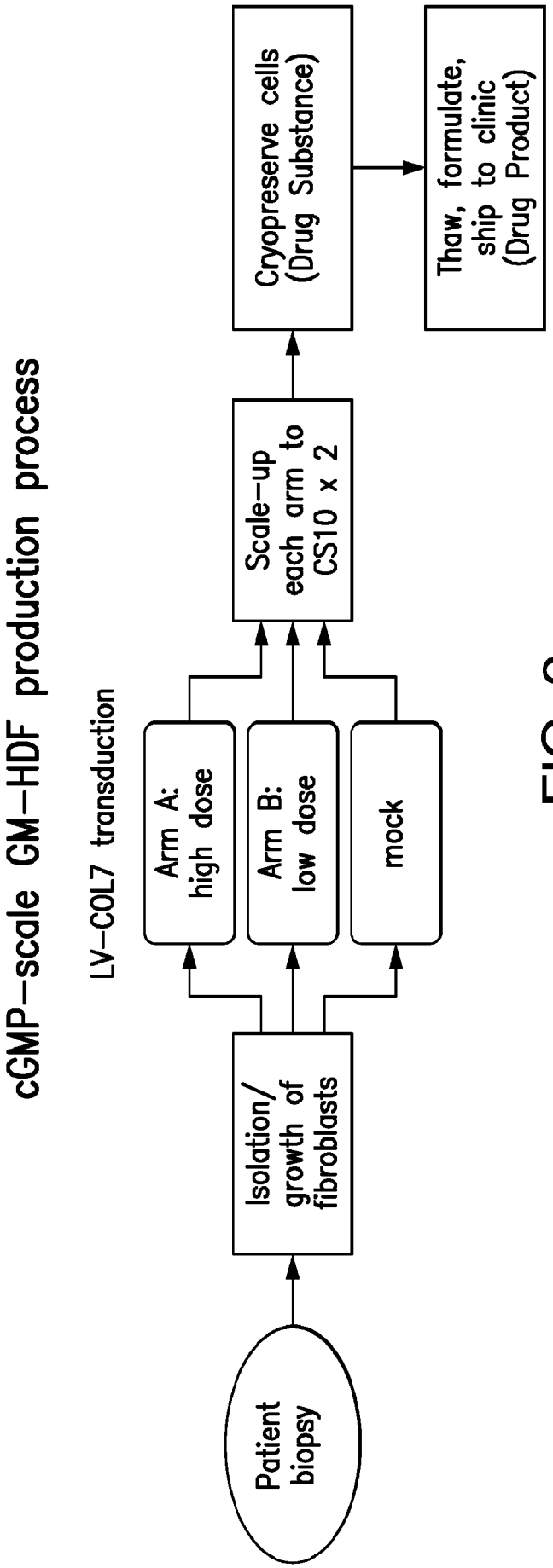

cGMP–scale GM–HDF production process
Patient biopsy
Isolation/ growth of fibroblasts
LV–COL7 transduction
Arm A: high dose
Arm B: low dose
mock
Scale–up each arm to CS10 x 2
Cryopreserve cells (Drug Substance)
Thaw, formulate, ship to clinic (Drug Product)

FIG. 2

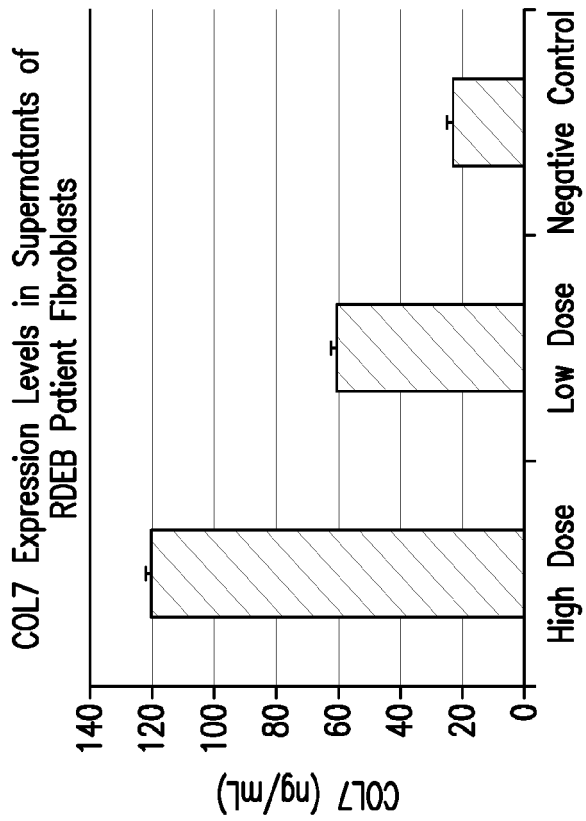

COL7 Expression Levels in Supernatants of RDEB Patient Fibroblasts
COL7 (ng/mL)
140
120
100
80
60
40
20
0
High Dose
Low Dose
Negative Control
Transduction Arm

FIG. 3B

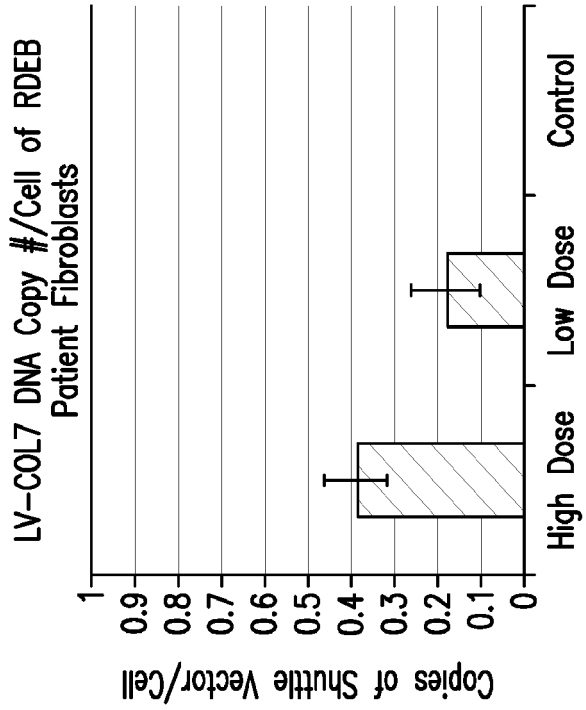

LV-COL7 DNA Copy #/Cell of RDEB Patient Fibroblasts
Copies of Shuttle Vector/Cell
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
High Dose
Low Dose
Control
Transduction Arm

FIG. 3A

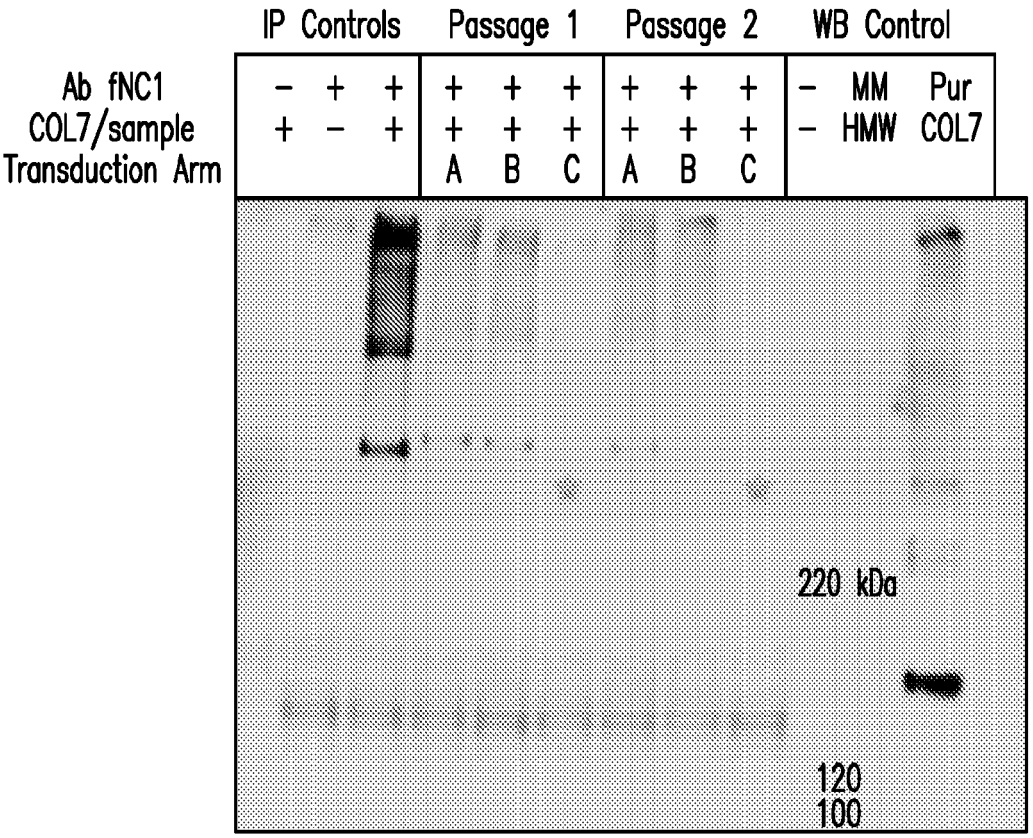

IP Controls
Passage 1
Passage 2
WB Control
Ab fNC1
COL7/sample
Transduction Arm
- + +
+ - +
+ + +
+ + +
A B C
+ + +
+ + +
A B C
- MM Pur
- HMW COL7
220 kDa
120
100

FIG. 3C

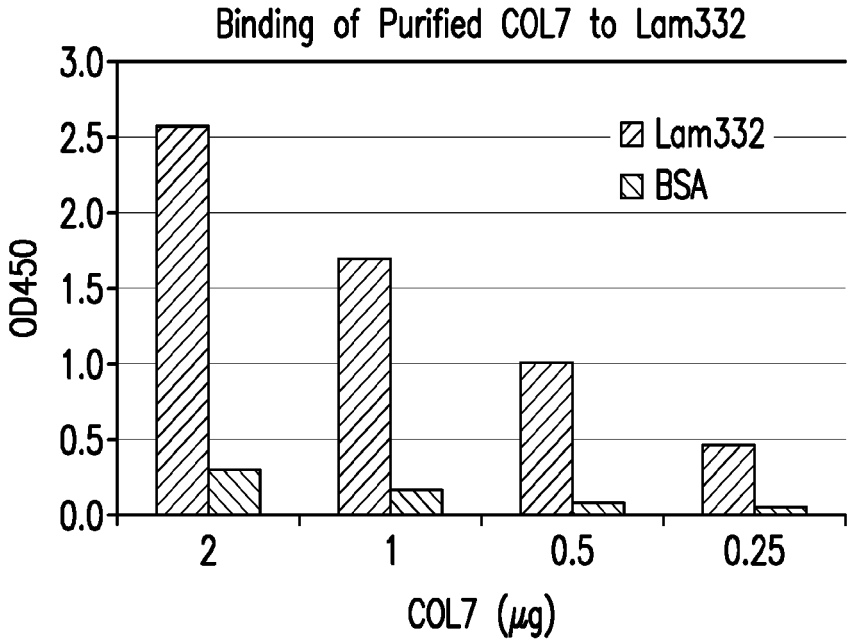

Binding of Purified COL7 to Lam332
3.0
2.5
2.0
1.5
1.0
0.5
0.0
OD450
Lam332
BSA
2
1
0.5
0.25
COL7 (μg)

FIG. 4A

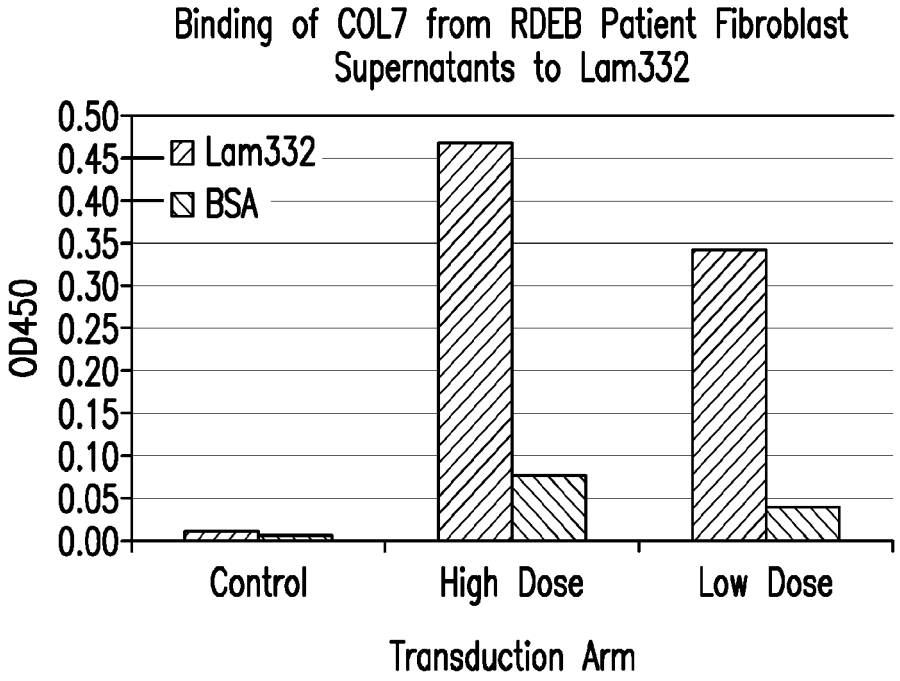

Binding of COL7 from RDEB Patient Fibroblast Supernatants to Lam332
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
OD450
Lam332
BSA
Control
High Dose
Low Dose
Transduction Arm

FIG. 4B

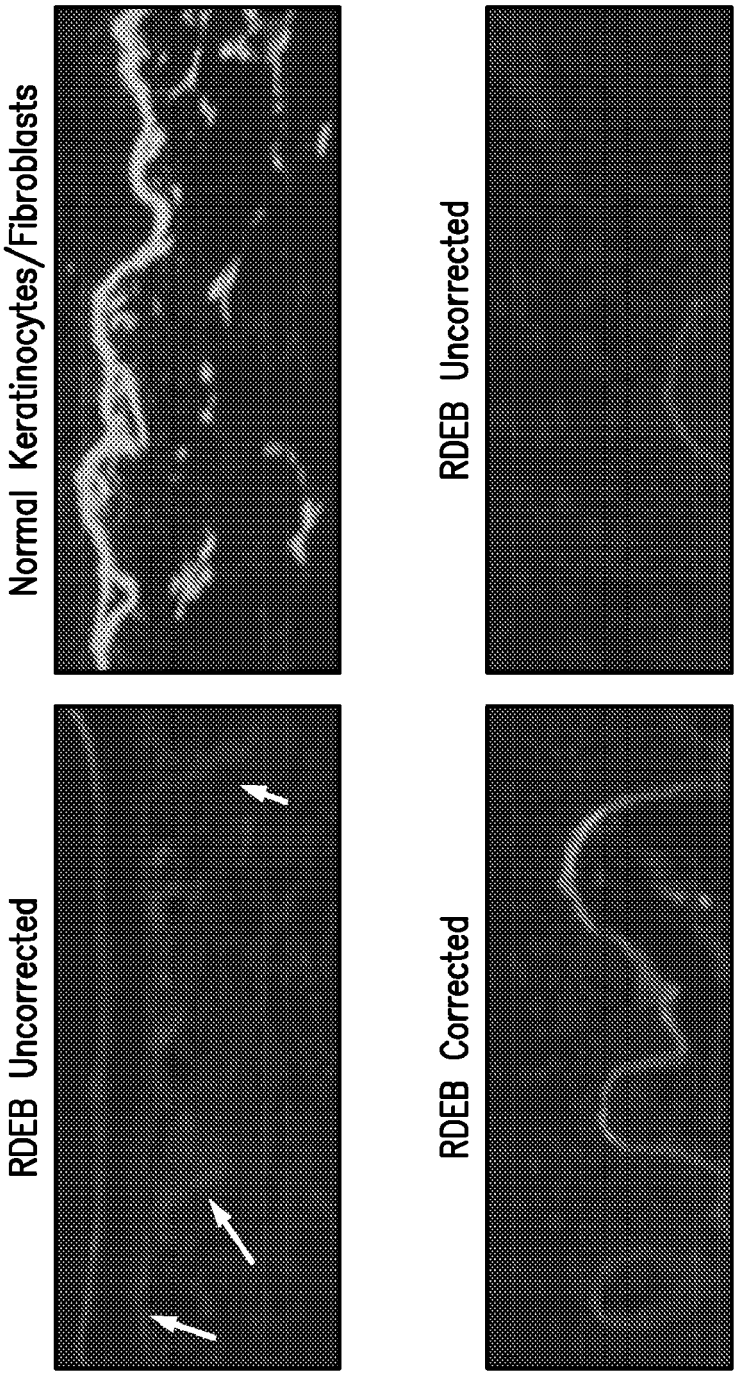

Normal Keratinocytes/Fibroblasts
RDEB Uncorrected
RDEB Uncorrected
RDEB Corrected

FIG. 5

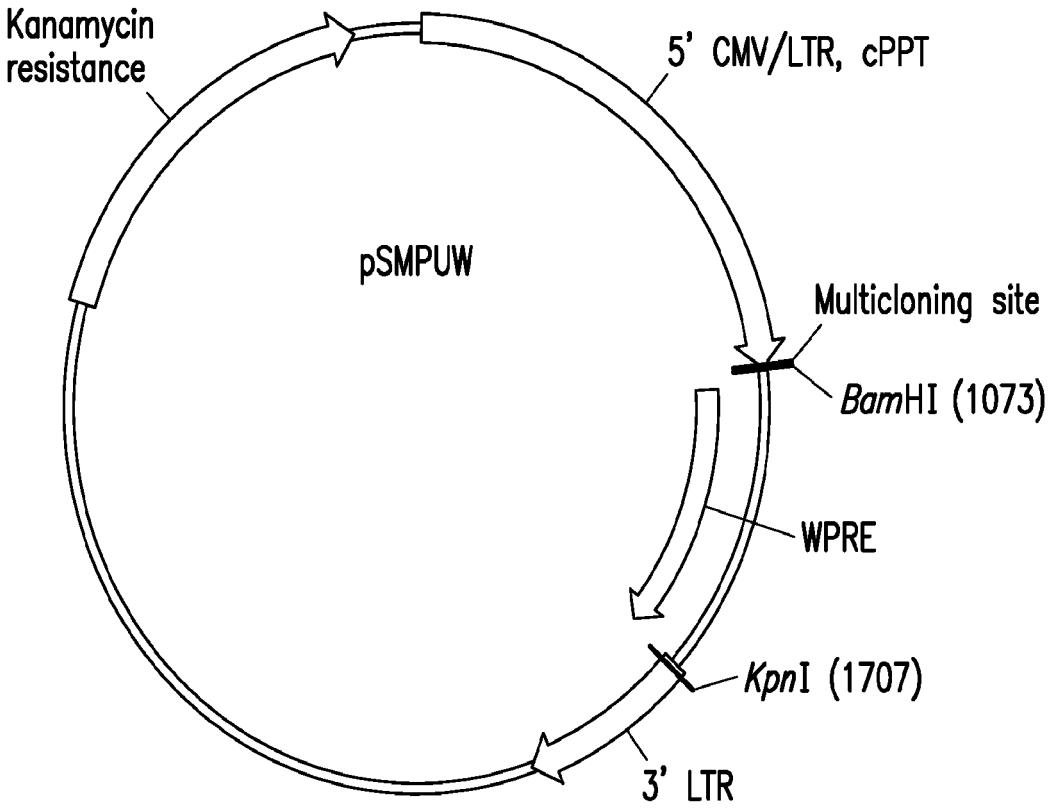

Kanamycin resistance
5' CMV/LTR, cPPT
pSMPUW
Multicloning site
BamHI (1073)
WPRE
KpnI (1707)
3' LTR

FIG. 6

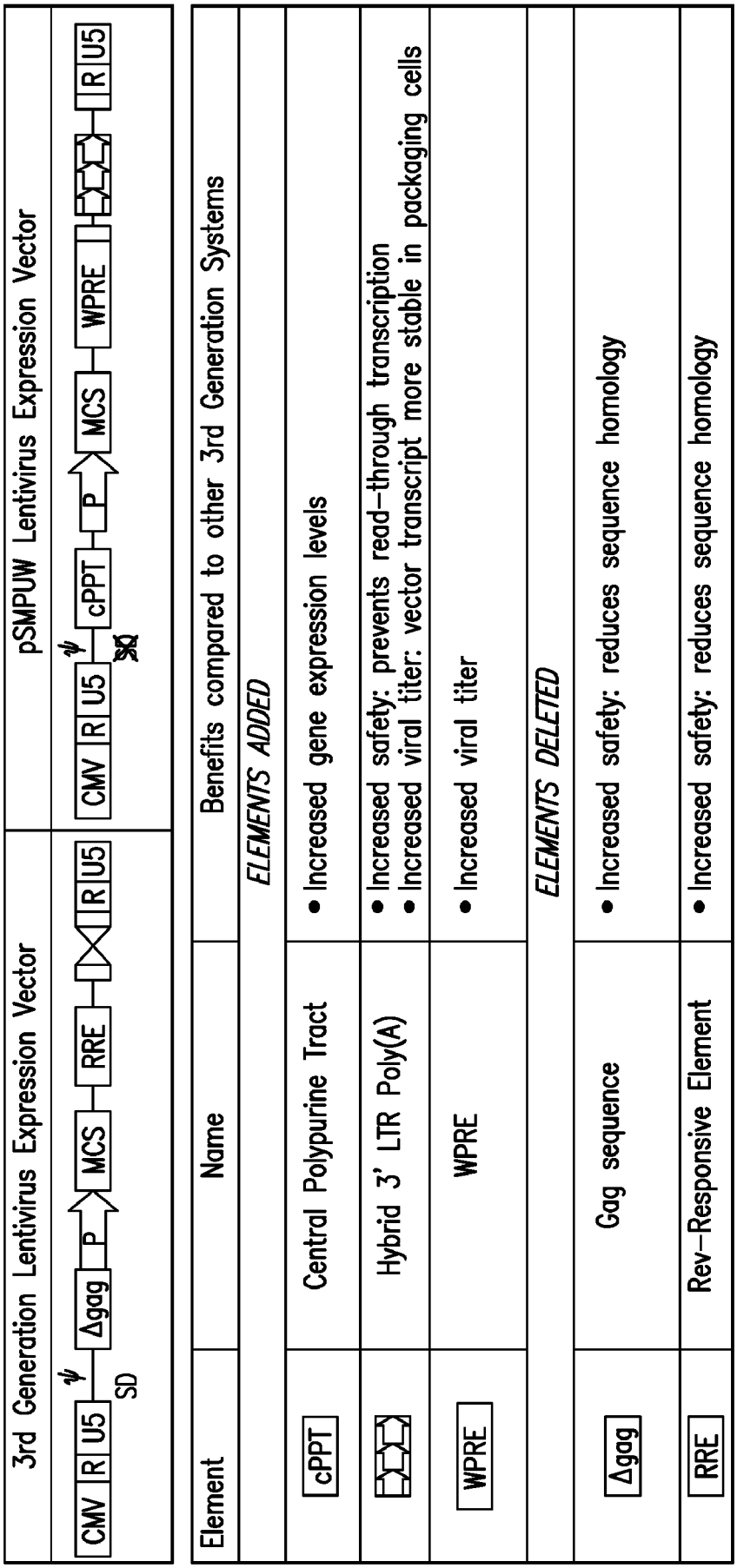

3rd Generation Lentivirus Expression Vector
CMV R U5 ψ SD Δgag P MCS RRE R U5
pSMPUW Lentivirus Expression Vector
CMV R U5 ψ SD cPPT P MCS WPRE R U5
Element
Name
Benefits compared to other 3rd Generation Systems
ELEMENTS ADDED
cPPT
Central Polypurine Tract
• Increased gene expression levels
Hybrid 3' LTR Poly(A)
• Increased safety: prevents read-through transcription
• Increased viral titer: vector transcript more stable in packaging cells
WPRE
WPRE
• Increased viral titer
ELEMENTS DELETED
Δgag
Gag sequence
• Increased safety: reduces sequence homology
RRE
Rev-Responsive Element
• Increased safety: reduces sequence homology

FIG. 7

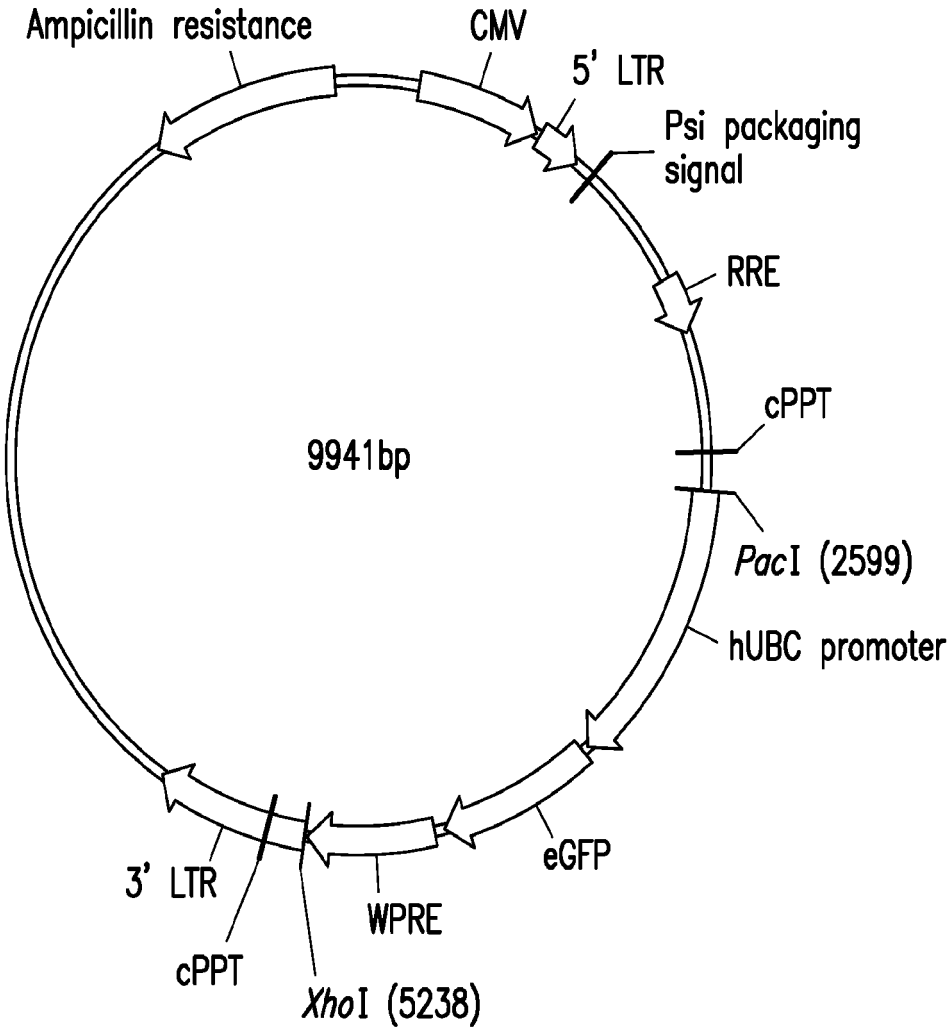
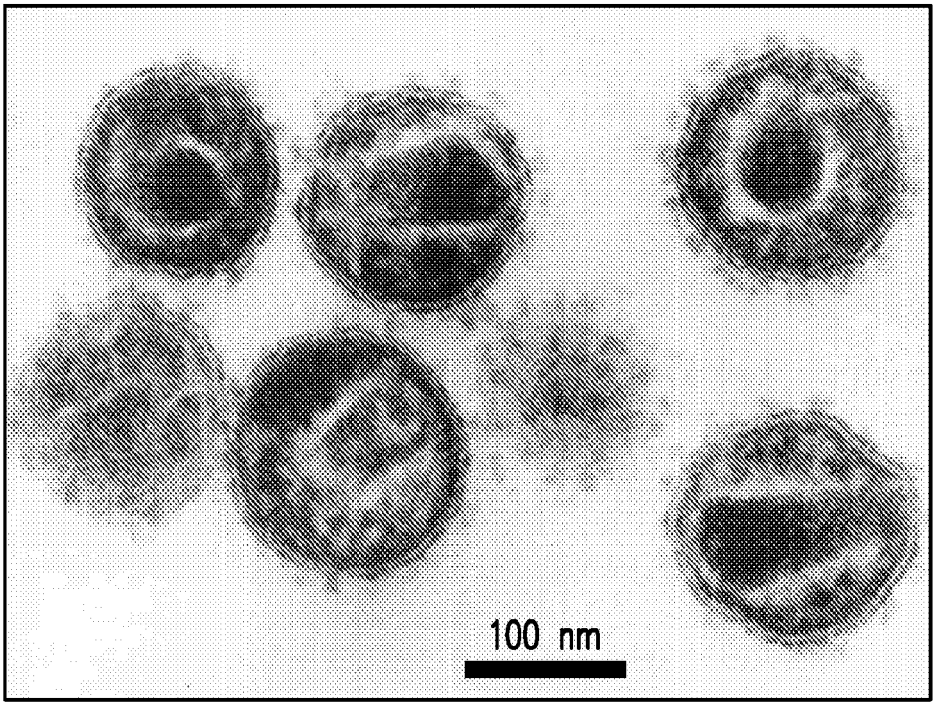

Ampicillin resistance
CMV
5' LTR
Psi packaging signal
RRE
cPPT
9941bp
PacI (2599)
hUBC promoter
eGFP
WPRE
3' LTR
cPPT
XhoI (5238)

FIG. 8



(Tomé 2013)

FIG. 9

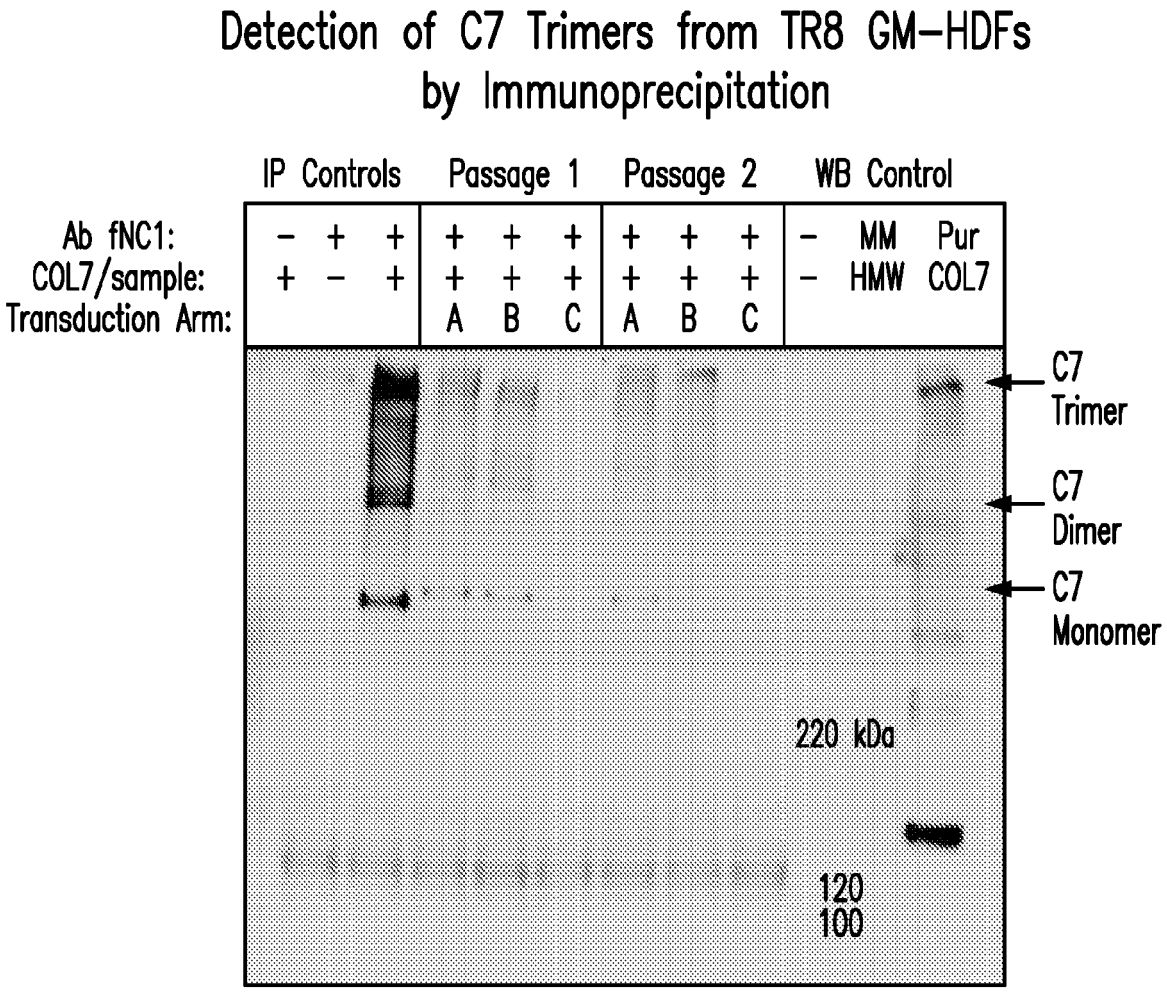

Detection of C7 Trimers from TR8 GM-HDFs
by Immunoprecipitation
IP Controls
Passage 1
Passage 2
WB Control
Ab fNC1:
COL7/sample:
Transduction Arm:
- + +
+ - +
+ + +
+ + +
A B C
A B C
- MM Pur
- HMW COL7
C7 Trimer
C7 Dimer
C7 Monomer
220 kDa
120
100

FIG. 10

Detection of C7 Trimers from TR10 and ER1 FCX-007 by Immunoprecipitation

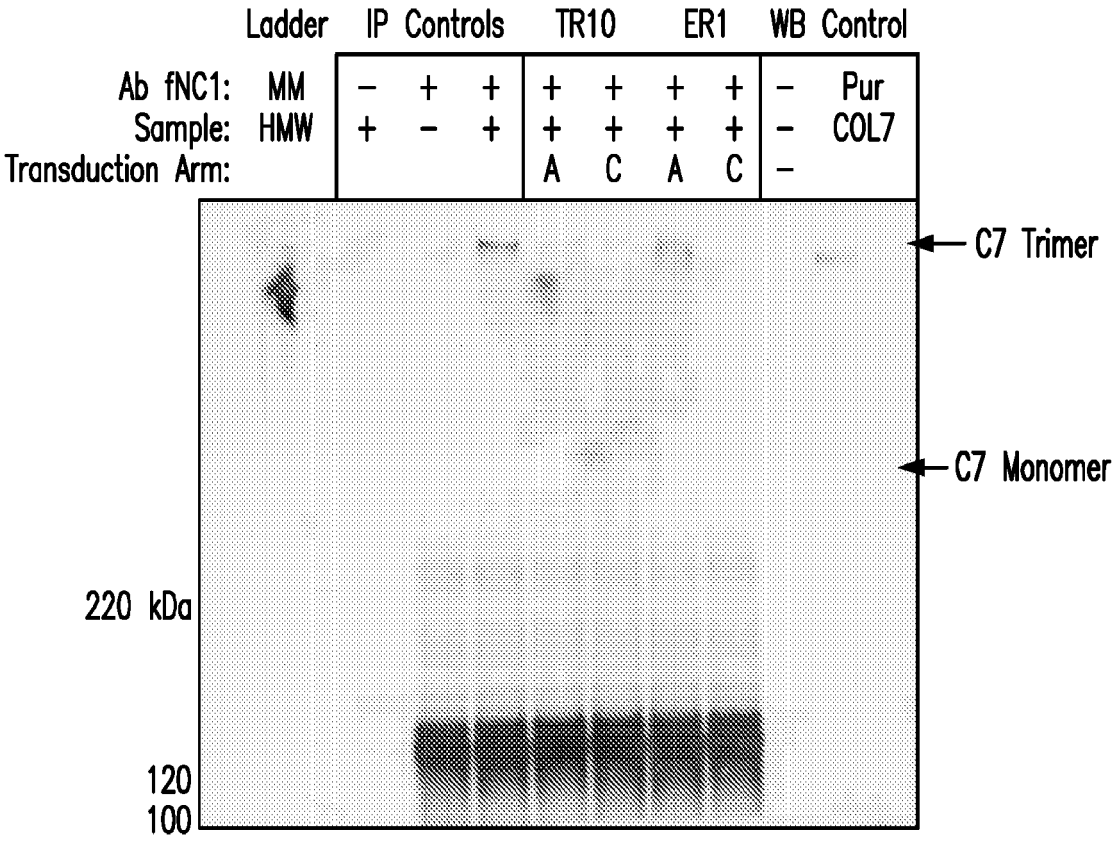


FIG. 11A

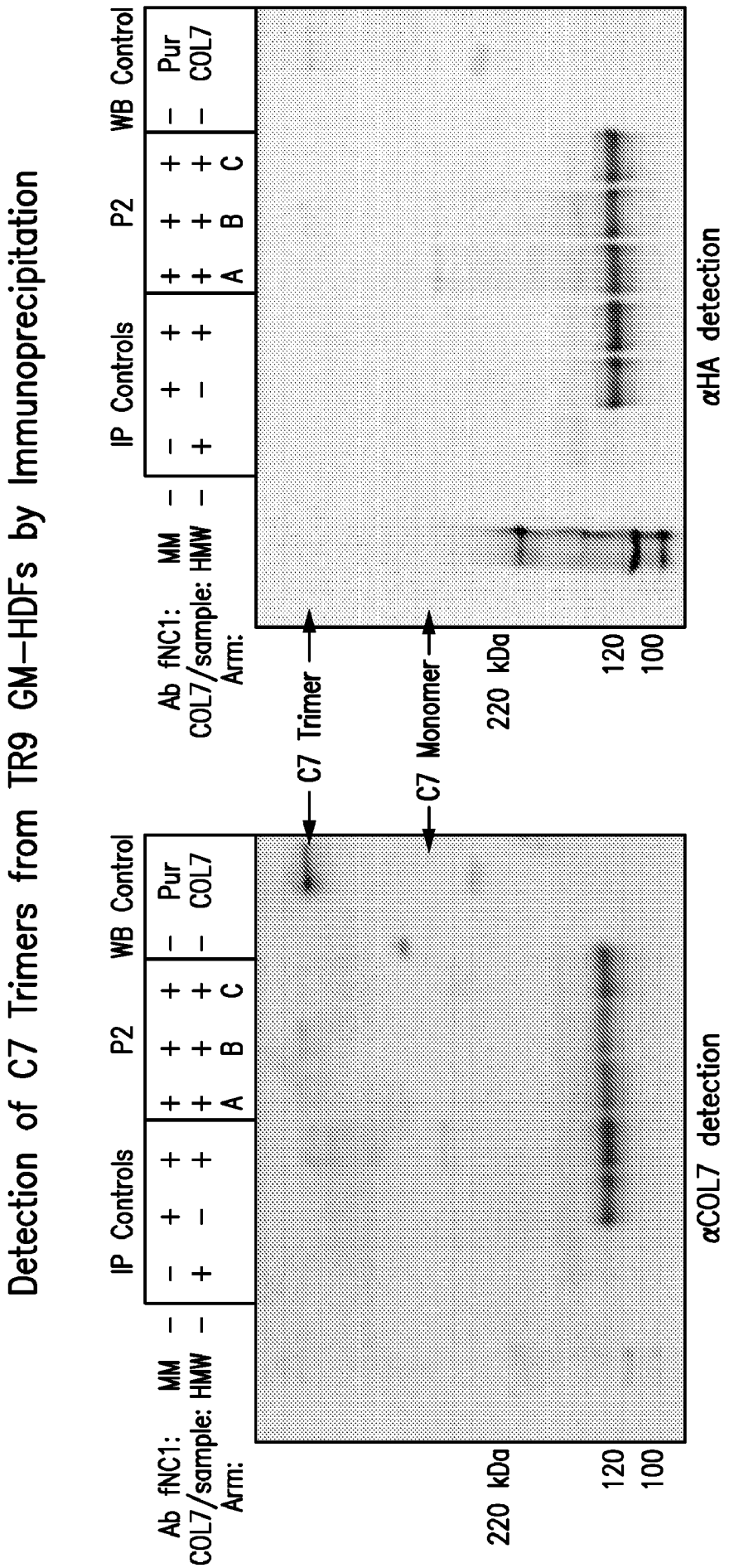

Detection of C7 Trimers from TR9 GM–HDFs by Immunoprecipitation
Ab fNC1: MM – + + +
COL7/sample: HMW – + – +
Arm: A B C
IP Controls
P2
WB Control
– Pur
– COL7
C7 Trimer
C7 Monomer
220 kDa
120
100
αCOL7 detection
αHA detection

FIG. 11B

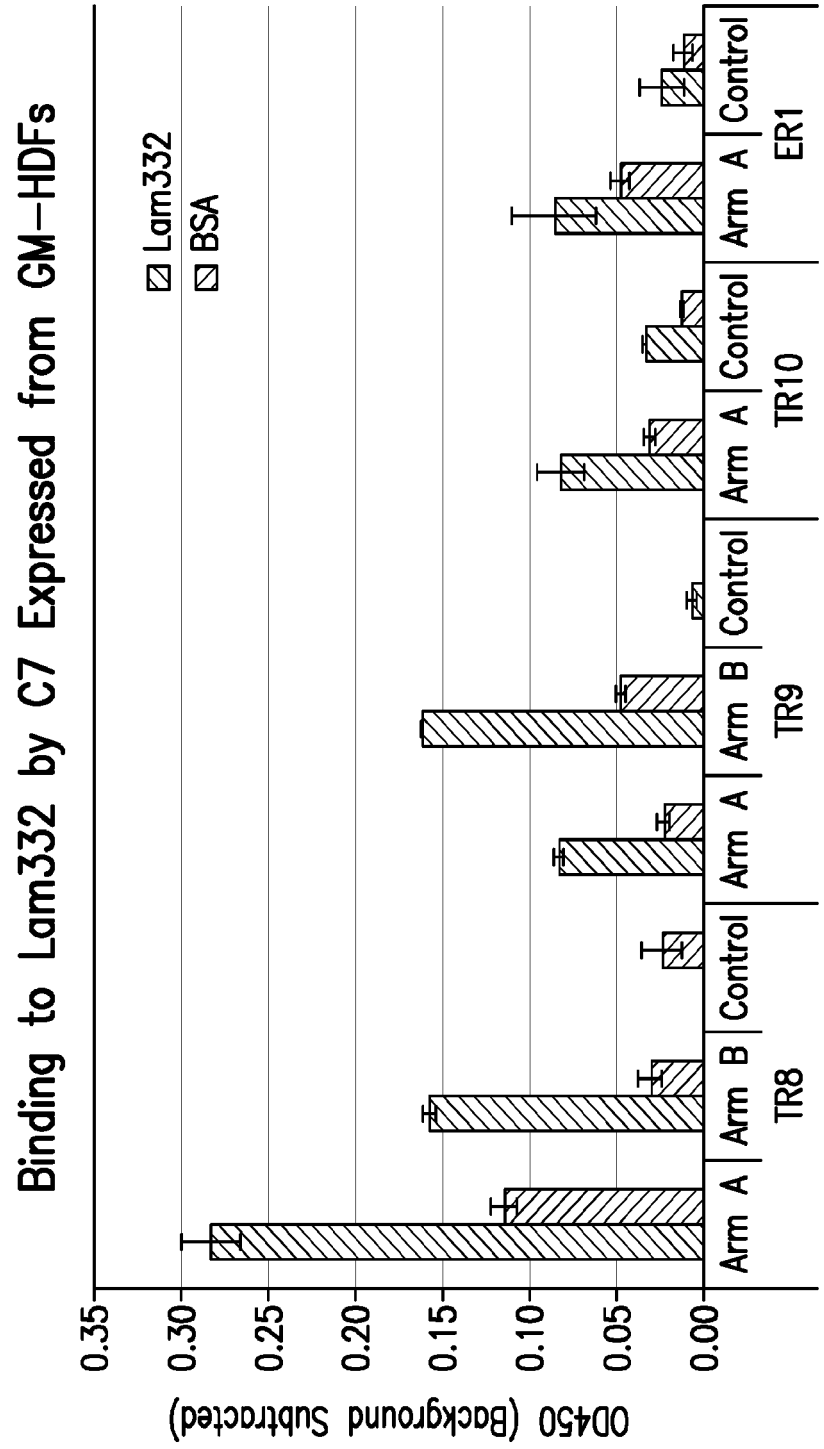

Binding to Lam332 by C7 Expressed from GM-HDFs
Lam332
BSA
OD450 (Background Subtracted)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Arm A
Arm B
Control
Arm A
Arm B
Control
Arm A
Control
Arm A
Control
TR8
TR9
TR10
ER1

FIG. 12

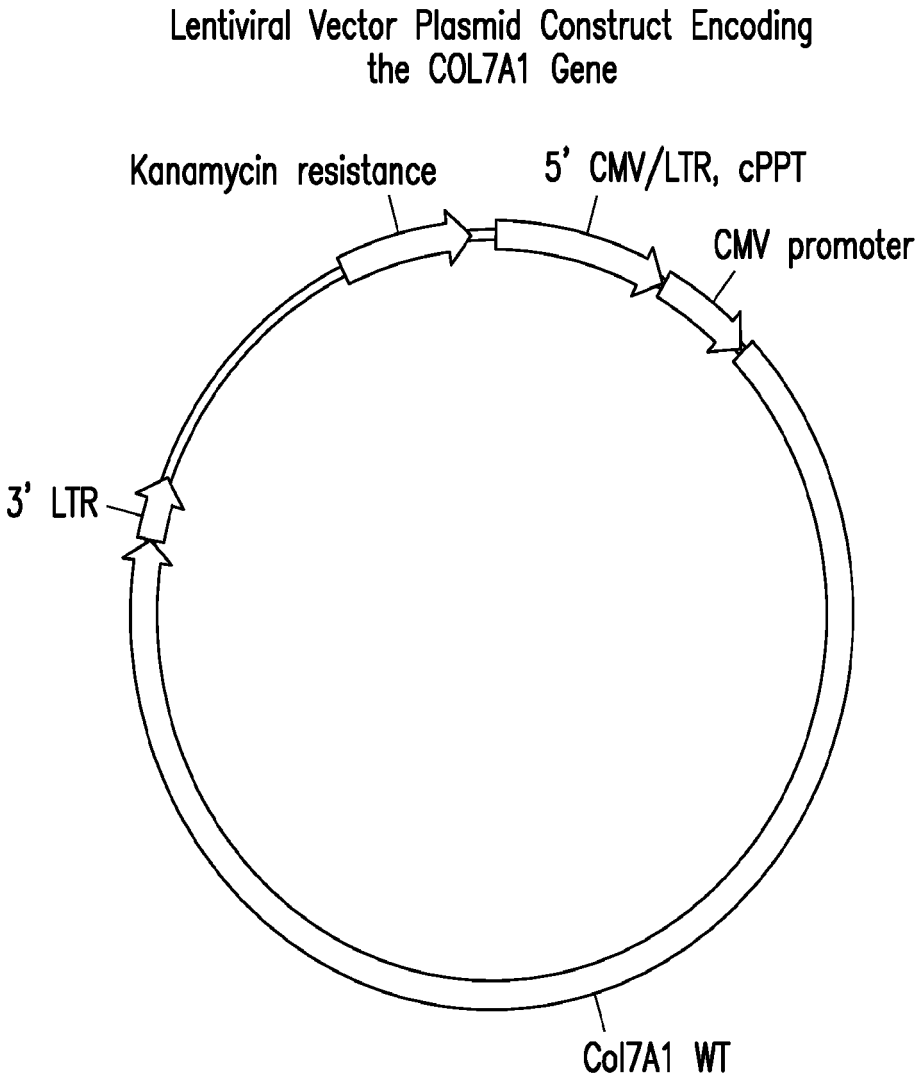

Lentiviral Vector Plasmid Construct Encoding the COL7A1 Gene
Kanamycin resistance
5' CMV/LTR, cPPT
CMV promoter
3' LTR
Col7A1 WT

FIG. 13

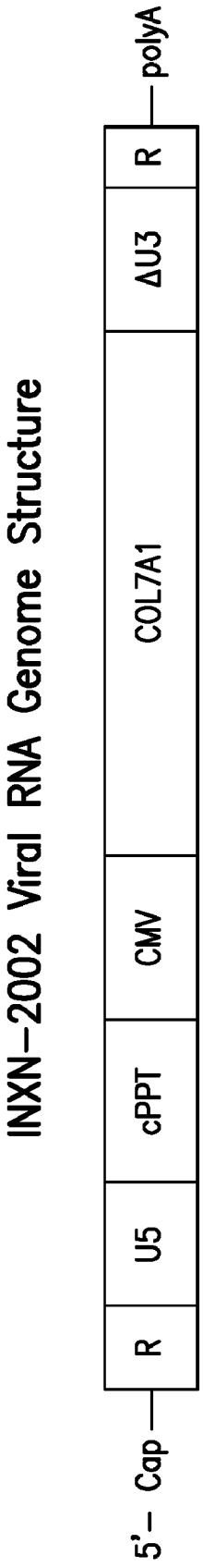

INXN-2002 Viral RNA Genome Structure
5'- Cap
R
U5
cPPT
CMV
COL7A1
ΔU3
R
polyA

FIG. 14

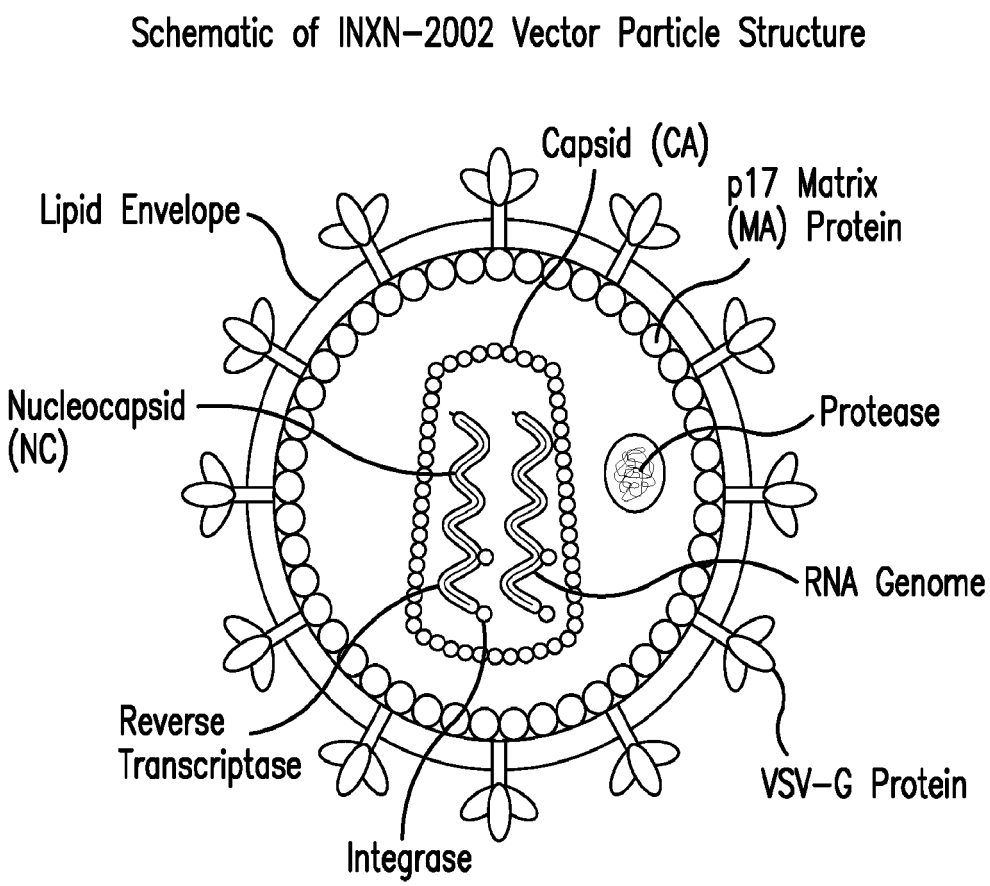

Schematic of INXN-2002 Vector Particle Structure
Capsid (CA)
Lipid Envelope
p17 Matrix
(MA) Protein
Nucleocapsid
(NC)
Protease
RNA Genome
Reverse
Transcriptase
VSV-G Protein
Integrase

FIG. 15

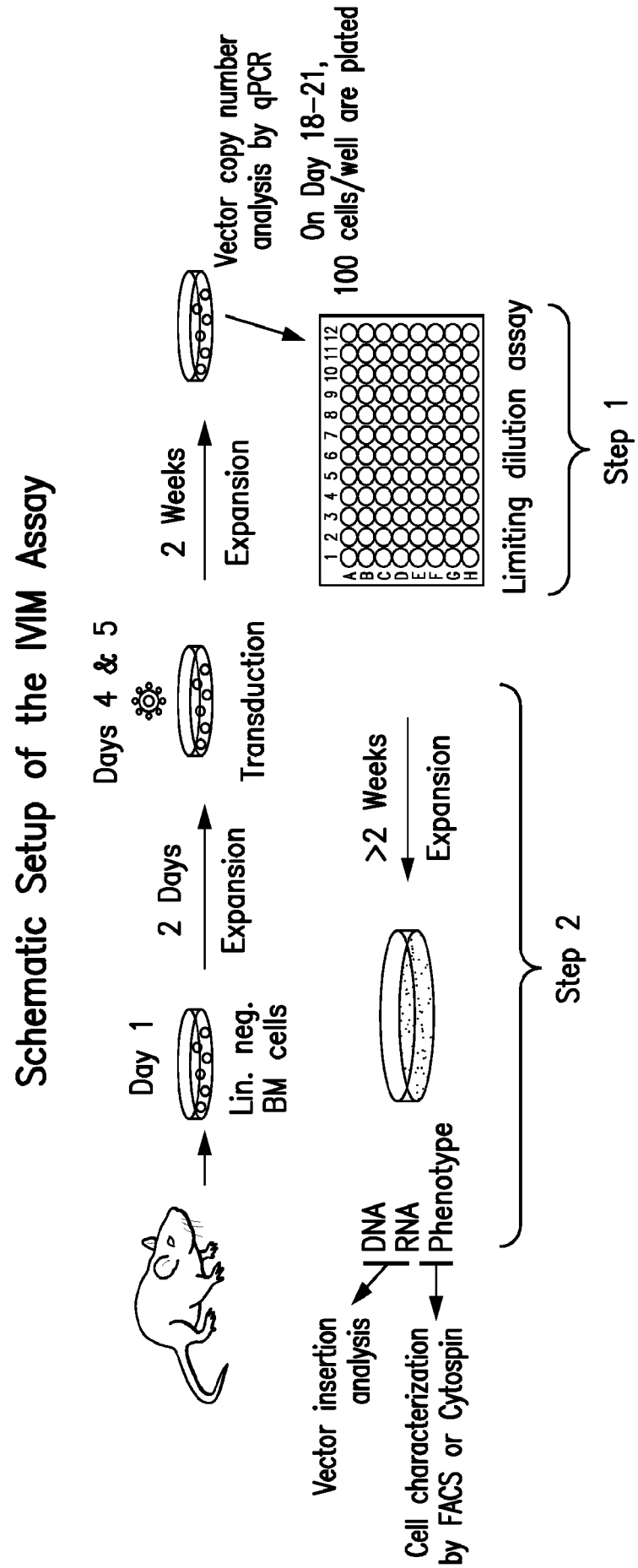

Schematic Setup of the IVIM Assay
Day 1
Lin. neg. BM cells
2 Days
Expansion
Days 4 & 5
Transduction
>2 Weeks
Expansion
2 Weeks
Expansion
Vector copy number analysis by qPCR
On Day 18–21, 100 cells/well are plated
Limiting dilution assay
Step 1
Step 2
DNA
RNA
Phenotype
Vector insertion analysis
Cell characterization by FACS or Cytospin

FIG. 16

Morphology and Structure of HDF Cells in Culture

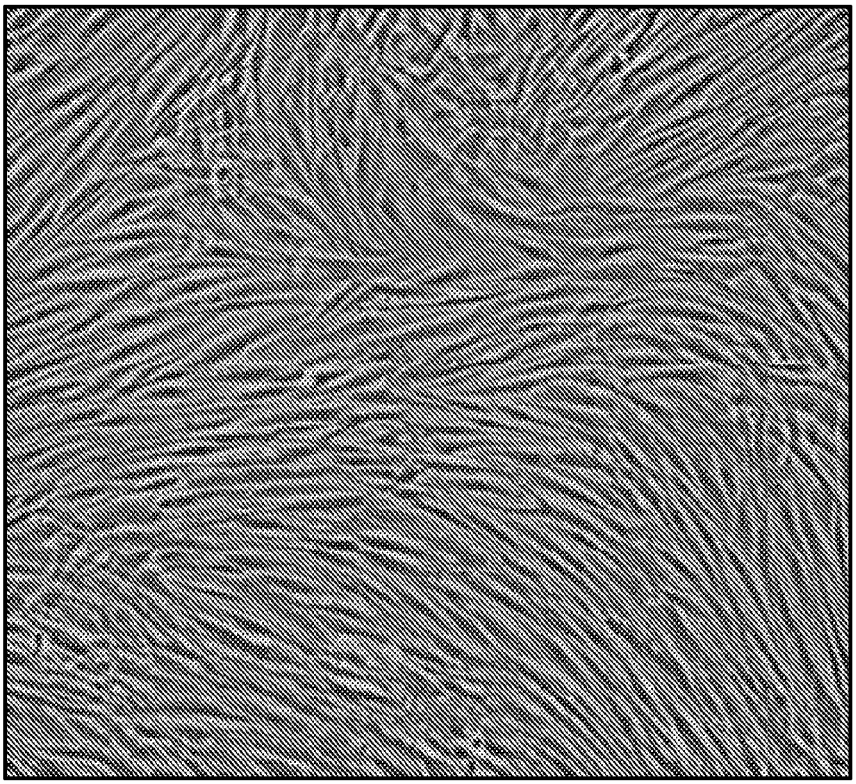


FIG. 17

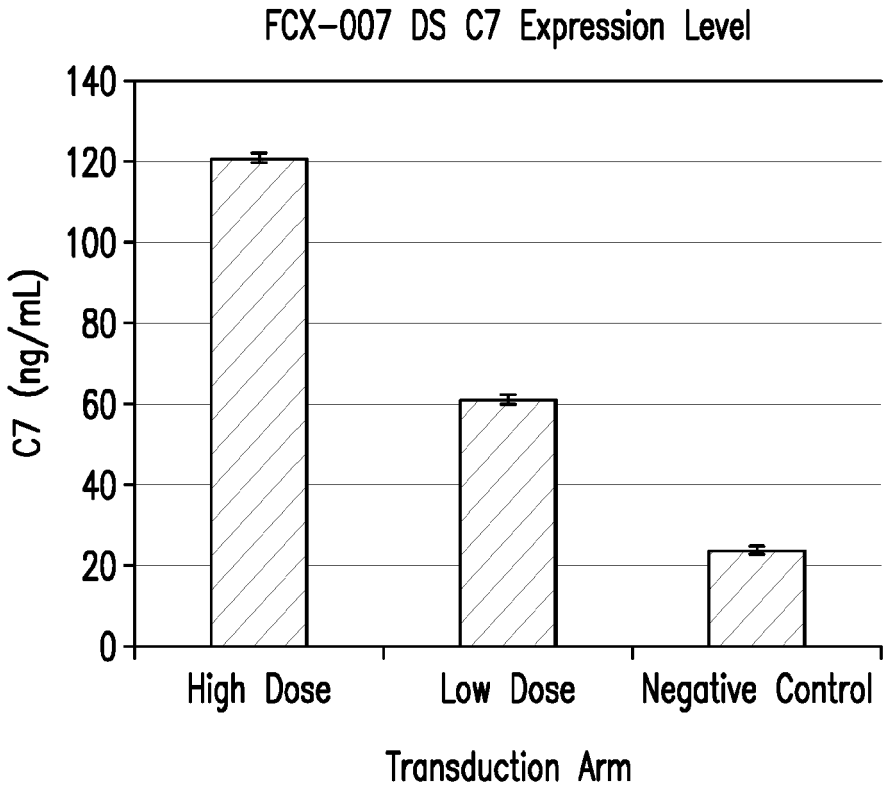

FCX-007 DS C7 Expression Level
140
120
100
80
60
40
20
0
C7 (ng/mL)
High Dose
Low Dose
Negative Control
Transduction Arm

FIG. 18

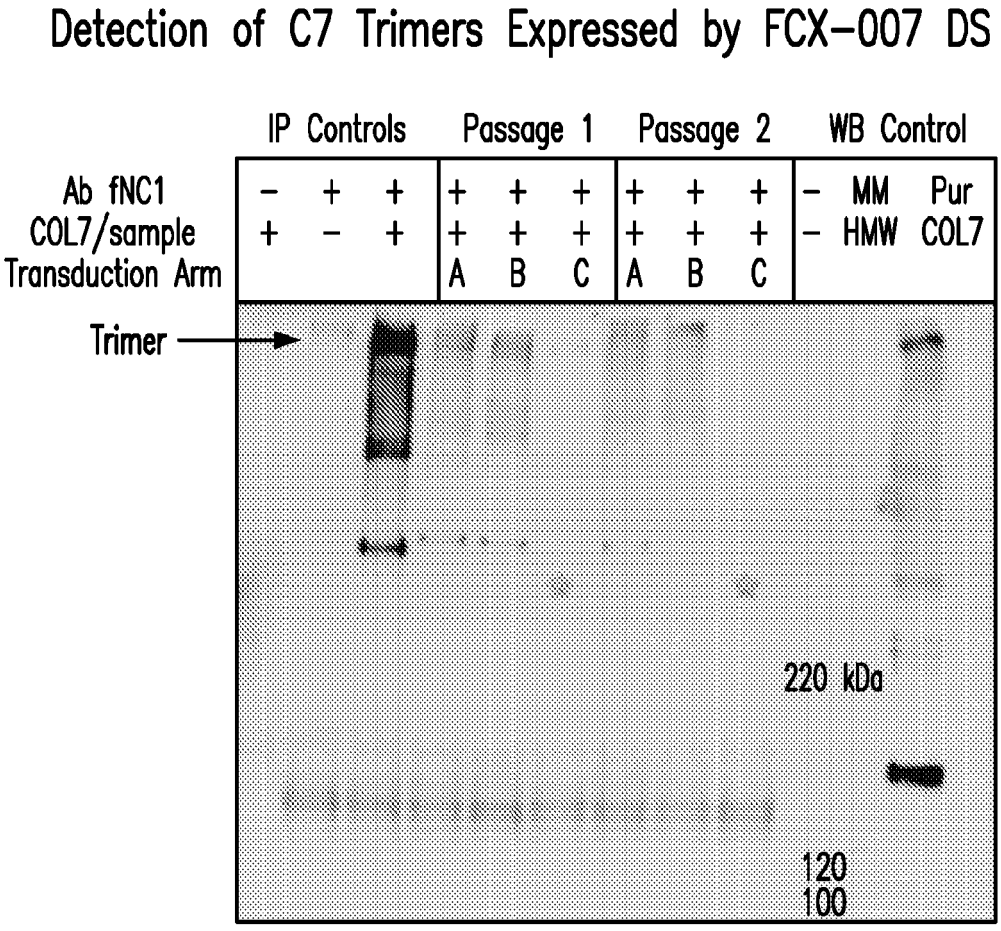

Detection of C7 Trimers Expressed by FCX-007 DS
IP Controls
Passage 1
Passage 2
WB Control
Ab fNC1
COL7/sample
Transduction Arm
- + +
+ - +
+ + +
+ + +
A B C
+ + +
+ + +
A B C
- MM Pur
- HMW COL7
Trimer
220 kDa
120
100

FIG. 19

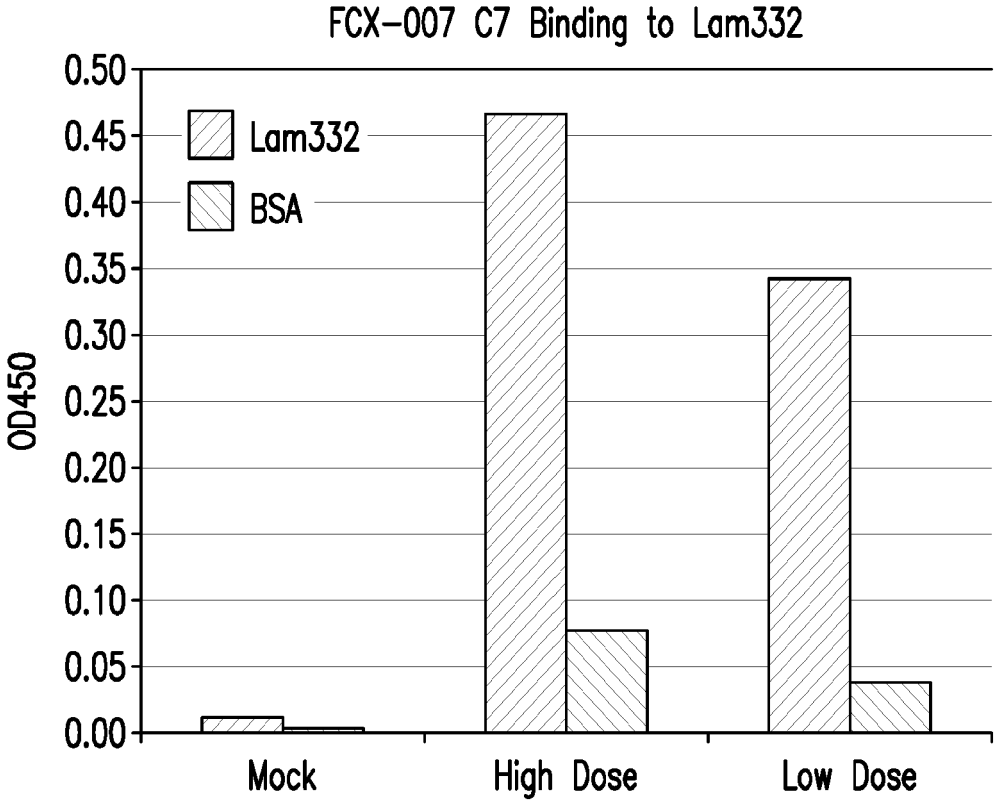

FCX-007 C7 Binding to Lam332
OD450
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Lam332
BSA
Mock
High Dose
Low Dose

FIG. 20

FCX-007 Migration Relative to NHDF and RDEB Fibroblasts

Migration of Normal and RDEB Patient Fibroblasts Transformed with LV-COL7

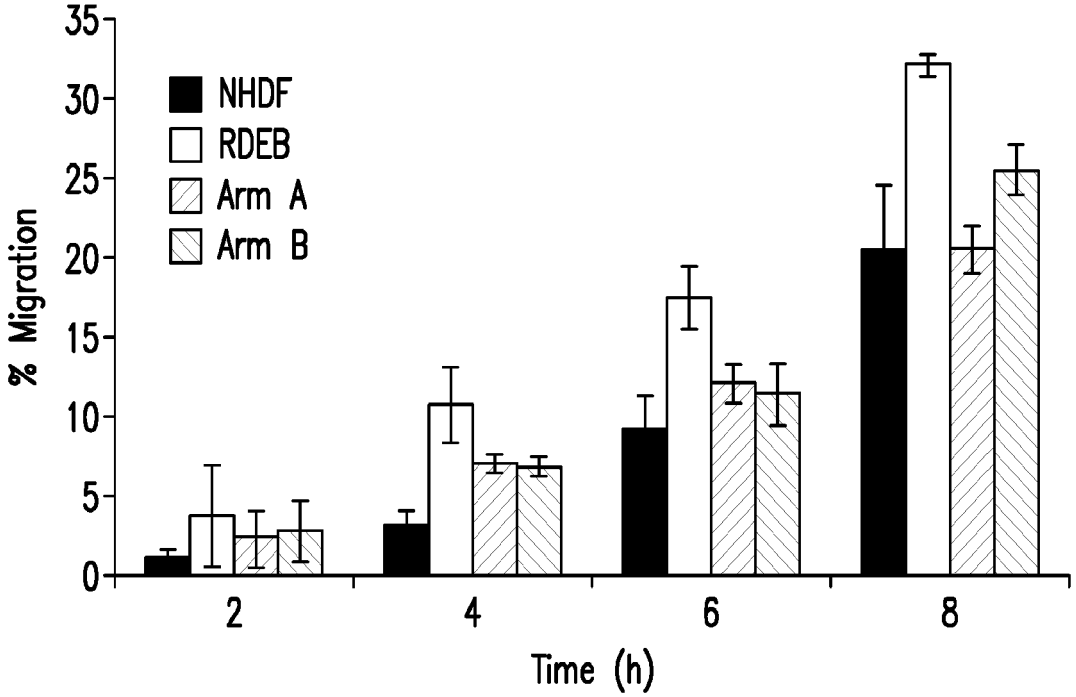


FIG. 21A

FCX-007 Migration Relative to NHDF and RDEB Fibroblasts

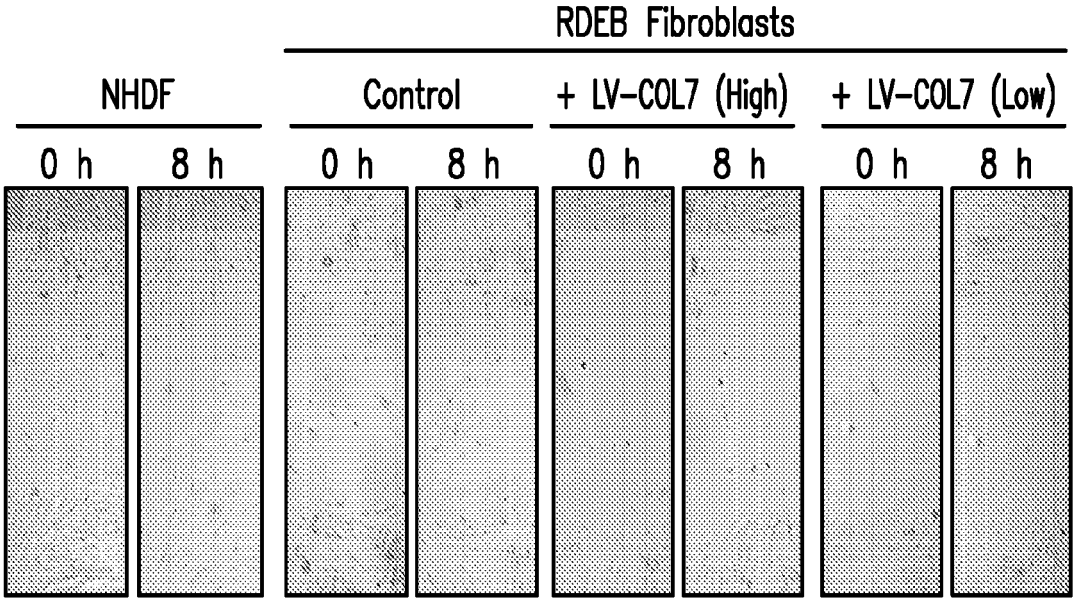


FIG. 21B

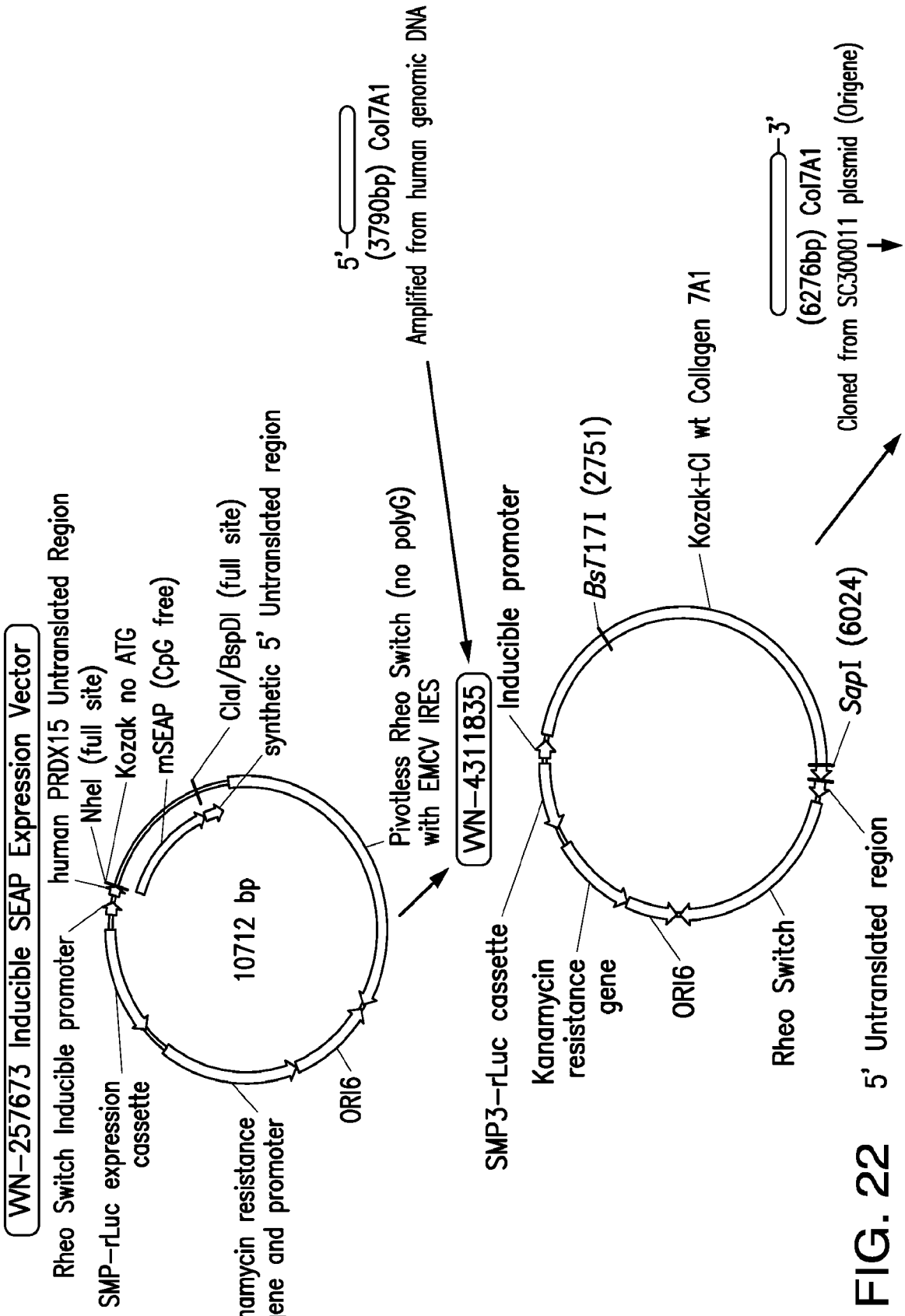

VN-257673 Inducible SEAP Expression Vector
Rheo Switch Inducible promoter
human PRDX15 Untranslated Region
NheI (full site)
Kozak no ATG
mSEAP (CpG free)
ClaI/BspDI (full site)
synthetic 5' Untranslated region
SMP-rLuc expression cassette
Kanamycin resistance gene and promoter
10712 bp
ORI6
Pivotless Rheo Switch (no polyG) with EMCV IRES
5'
(3790bp) Col7A1
Amplified from human genomic DNA
VN-4311835
Inducible promoter
SMP3-rLuc cassette
Kanamycin resistance gene
ORI6
Rheo Switch
5' Untranslated region
BsT171 (2751)
Kozak+Cl wt Collagen 7A1
SapI (6024)
3'
(6276bp) Col7A1
Cloned from SC300011 plasmid (Origene)

FIG. 22

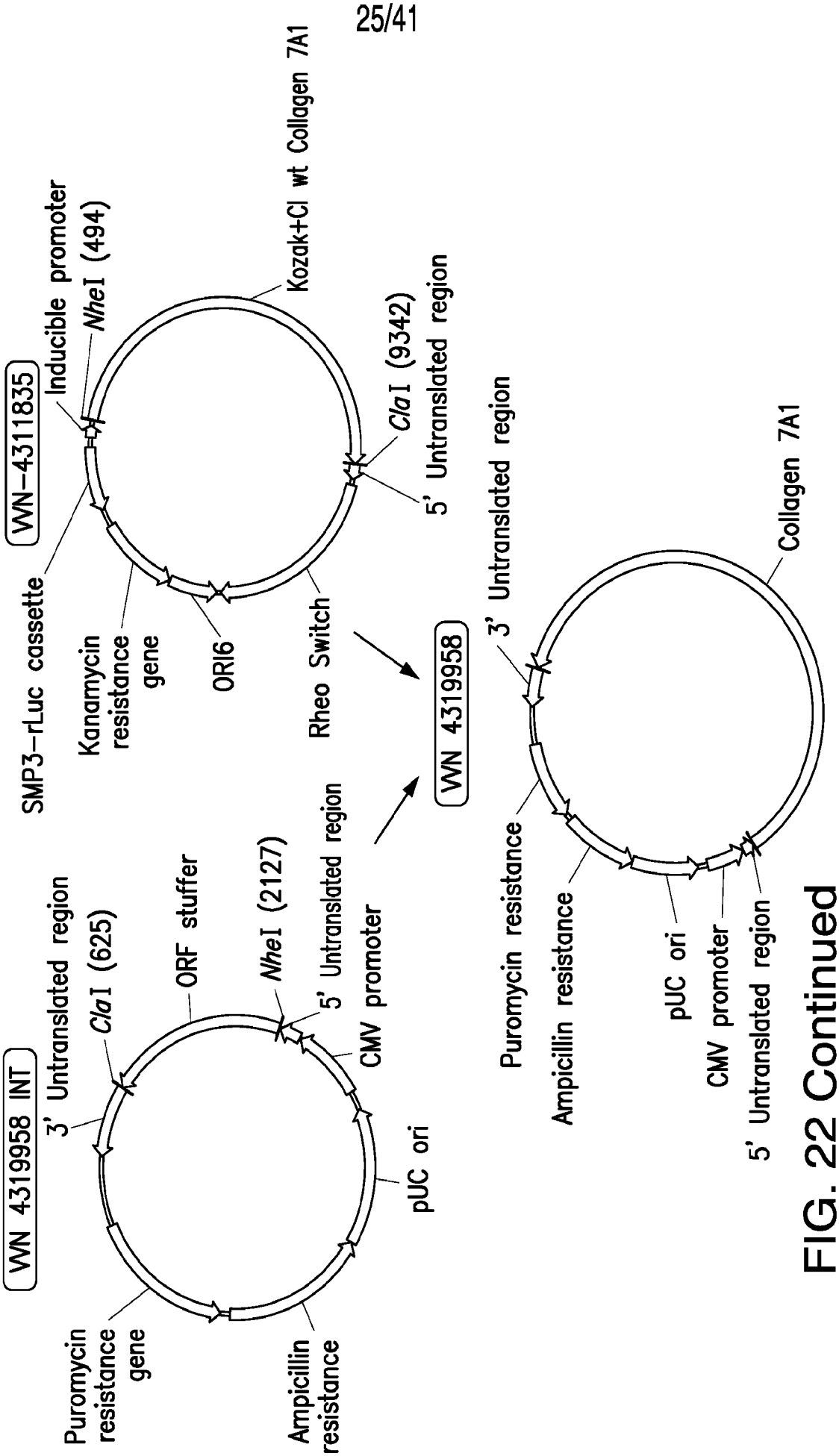


FIG. 22 Continued

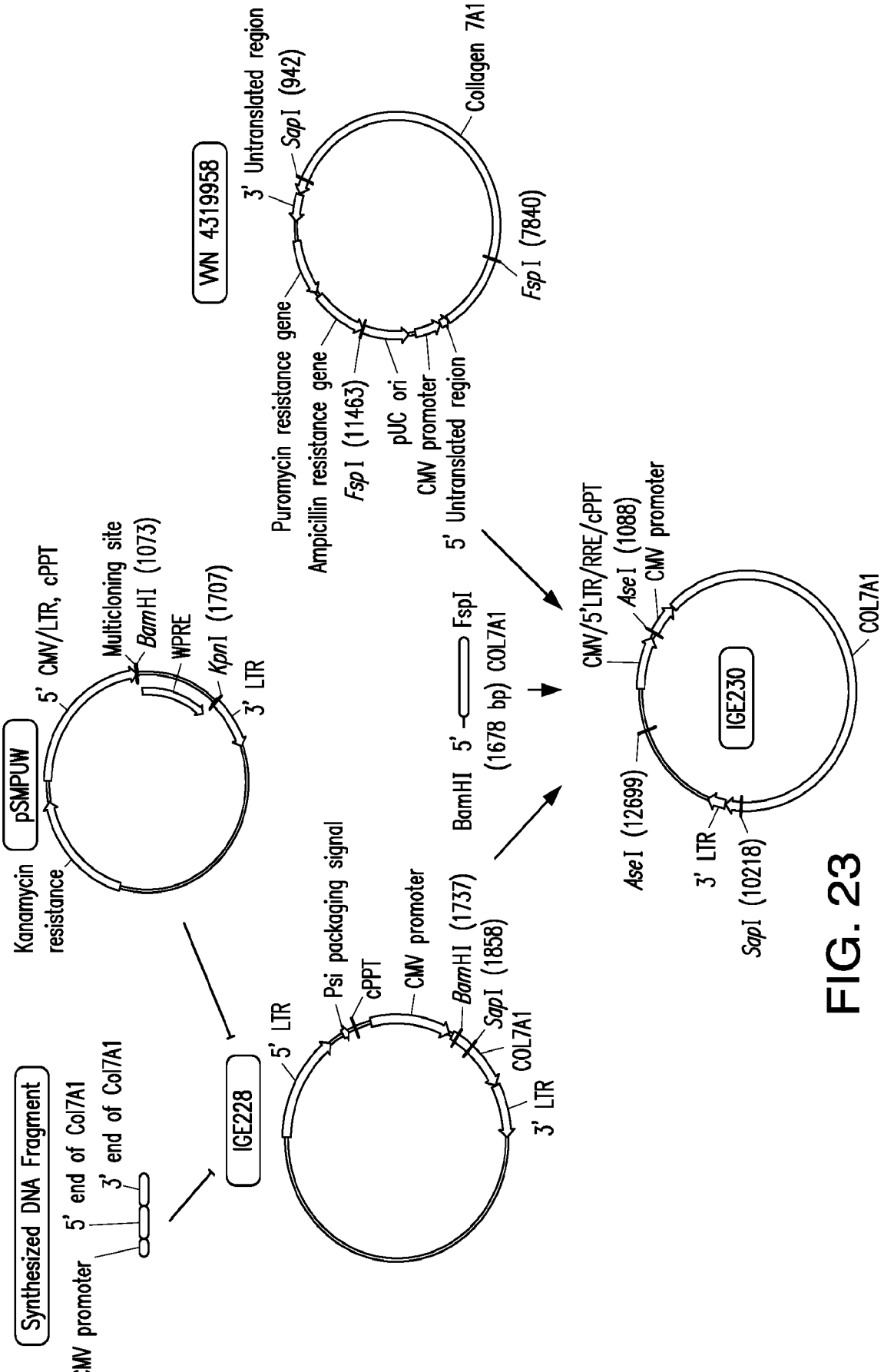

Synthesized DNA Fragment
CMV promoter
5' end of Col7A1
3' end of Col7A1
IGE228
5' LTR
Psi packaging signal
cPPT
CMV promoter
BamHI (1737)
SapI (1858)
COL7A1
3' LTR
BamHI 5'
FspI
(1678 bp) COL7A1
pSMPUW
Kanamycin resistance
5' CMV/LTR, cPPT
Multicloning site
BamHI (1073)
WPRE
KpnI (1707)
3' LTR
WN 4319958
3' Untranslated region
SapI (942)
Collagen 7A1
Puromycin resistance gene
Ampicillin resistance gene
FspI (11463)
pUC ori
CMV promoter
5' Untranslated region
FspI (7840)
CMV/5'LTR/RRE/cPPT
AseI (1088)
CMV promoter
IGE230
COL7A1
AseI (12699)
3' LTR
SapI (10218)

FIG. 23

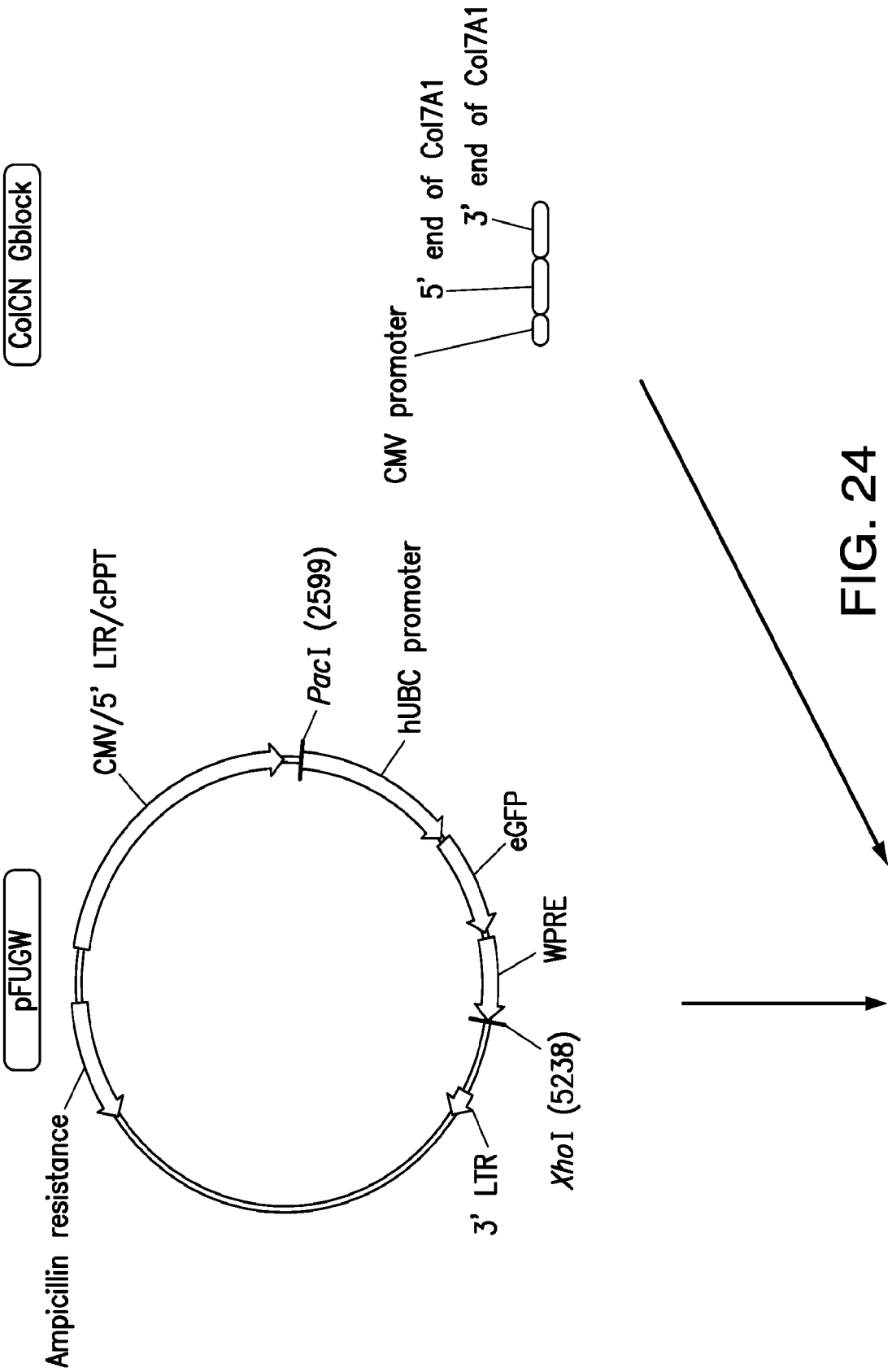

ColCN Gblock
CMV promoter
5' end of Col7A1
3' end of Col7A1
pFUGW
CMV/5' LTR/cPPT
PacI (2599)
hUBC promoter
eGFP
WPRE
XhoI (5238)
3' LTR
Ampicillin resistance

FIG. 24

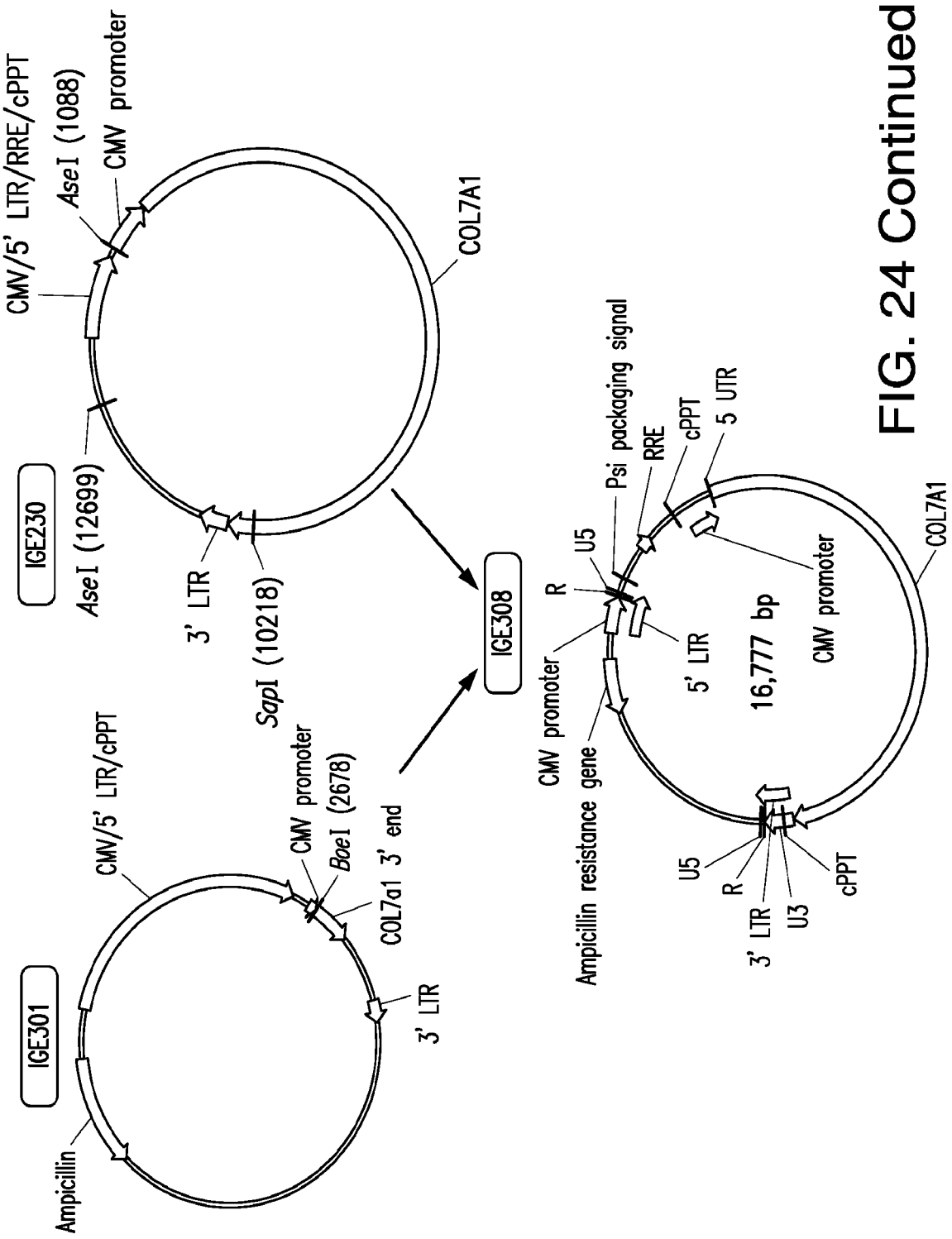


FIG. 24 Continued

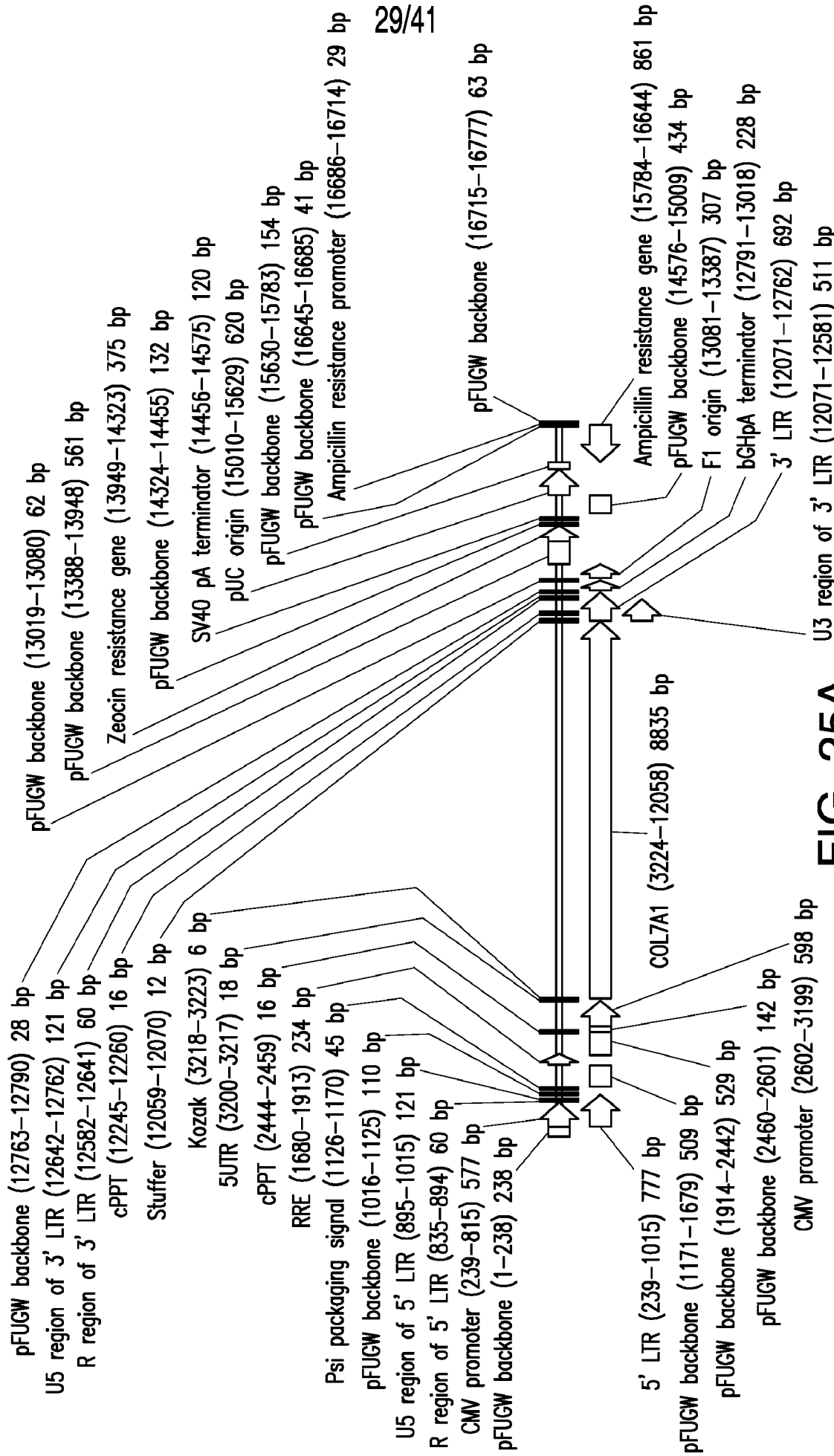

pFUGW backbone (12763–12790) 28 bp
U5 region of 3' LTR (12642–12762) 121 bp
R region of 3' LTR (12582–12641) 60 bp
cPPT (12245–12260) 16 bp
Stuffer (12059–12070) 12 bp
Kozak (3218–3223) 6 bp
5UTR (3200–3217) 18 bp
cPPT (2444–2459) 16 bp
RRE (1680–1913) 234 bp
Psi packaging signal (1126–1170) 45 bp
pFUGW backbone (1016–1125) 110 bp
U5 region of 5' LTR (895–1015) 121 bp
R region of 5' LTR (835–894) 60 bp
CMV promoter (239–815) 577 bp
pFUGW backbone (1–238) 238 bp
5' LTR (239–1015) 777 bp
pFUGW backbone (1171–1679) 509 bp
pFUGW backbone (1914–2442) 529 bp
pFUGW backbone (2460–2601) 142 bp
CMV promoter (2602–3199) 598 bp
COL7A1 (3224–12058) 8835 bp
pFUGW backbone (13019–13080) 62 bp
pFUGW backbone (13388–13948) 561 bp
Zeocin resistance gene (13949–14323) 375 bp
pFUGW backbone (14324–14455) 132 bp
SV40 pA terminator (14456–14575) 120 bp
pUC origin (15010–15629) 620 bp
pFUGW backbone (15630–15783) 154 bp
pFUGW backbone (16645–16685) 41 bp
Ampicillin resistance promoter (16686–16714) 29 bp
pFUGW backbone (16715–16777) 63 bp
Ampicillin resistance gene (15784–16644) 861 bp
pFUGW backbone (14576–15009) 434 bp
F1 origin (13081–13387) 307 bp
bGHpA terminator (12791–13018) 228 bp
3' LTR (12071–12762) 692 bp
U3 region of 3' LTR (12071–12581) 511 bp

FIG. 25A

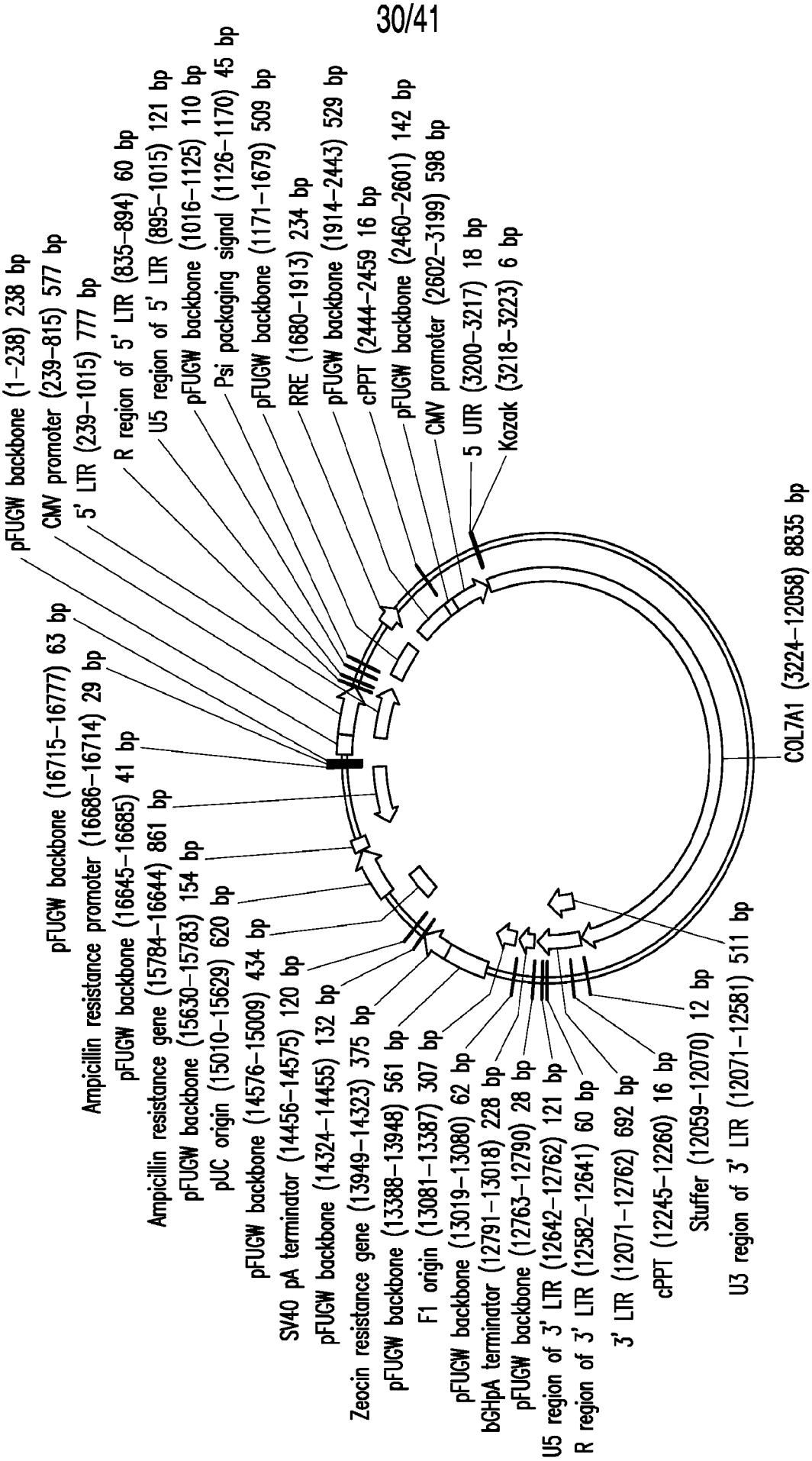

pFUGW backbone (1–238) 238 bp
CMV promoter (239–815) 577 bp
5' LTR (239–1015) 777 bp
R region of 5' LTR (835–894) 60 bp
U5 region of 5' LTR (895–1015) 121 bp
pFUGW backbone (1016–1125) 110 bp
Psi packaging signal (1126–1170) 45 bp
pFUGW backbone (1171–1679) 509 bp
RRE (1680–1913) 234 bp
pFUGW backbone (1914–2443) 529 bp
cPPT (2444–2459 16 bp
pFUGW backbone (2460–2601) 142 bp
CMV promoter (2602–3199) 598 bp
5 UTR (3200–3217) 18 bp
Kozak (3218–3223) 6 bp
COL7A1 (3224–12058) 8835 bp
U3 region of 3' LTR (12071–12581) 511 bp
Stuffer (12059–12070) 12 bp
cPPT (12245–12260) 16 bp
3' LTR (12071–12762) 692 bp
R region of 3' LTR (12582–12641) 60 bp
U5 region of 3' LTR (12642–12762) 121 bp
pFUGW backbone (12763–12790) 28 bp
bGHpA terminator (12791–13018) 228 bp
pFUGW backbone (13019–13080) 62 bp
F1 origin (13081–13387) 307 bp
pFUGW backbone (13388–13948) 561 bp
Zeocin resistance gene (13949–14323) 375 bp
pFUGW backbone (14324–14455) 132 bp
SV40 pA terminator (14456–14575) 120 bp
pFUGW backbone (14576–15009) 434 bp
pUC origin (15010–15629) 620 bp
pFUGW backbone (15630–15783) 154 bp
Ampicillin resistance gene (15784–16644) 861 bp
pFUGW backbone (16645–16685) 41 bp
Ampicillin resistance promoter (16686–16714) 29 bp
pFUGW backbone (16715–16777) 63 bp

FIG. 25B

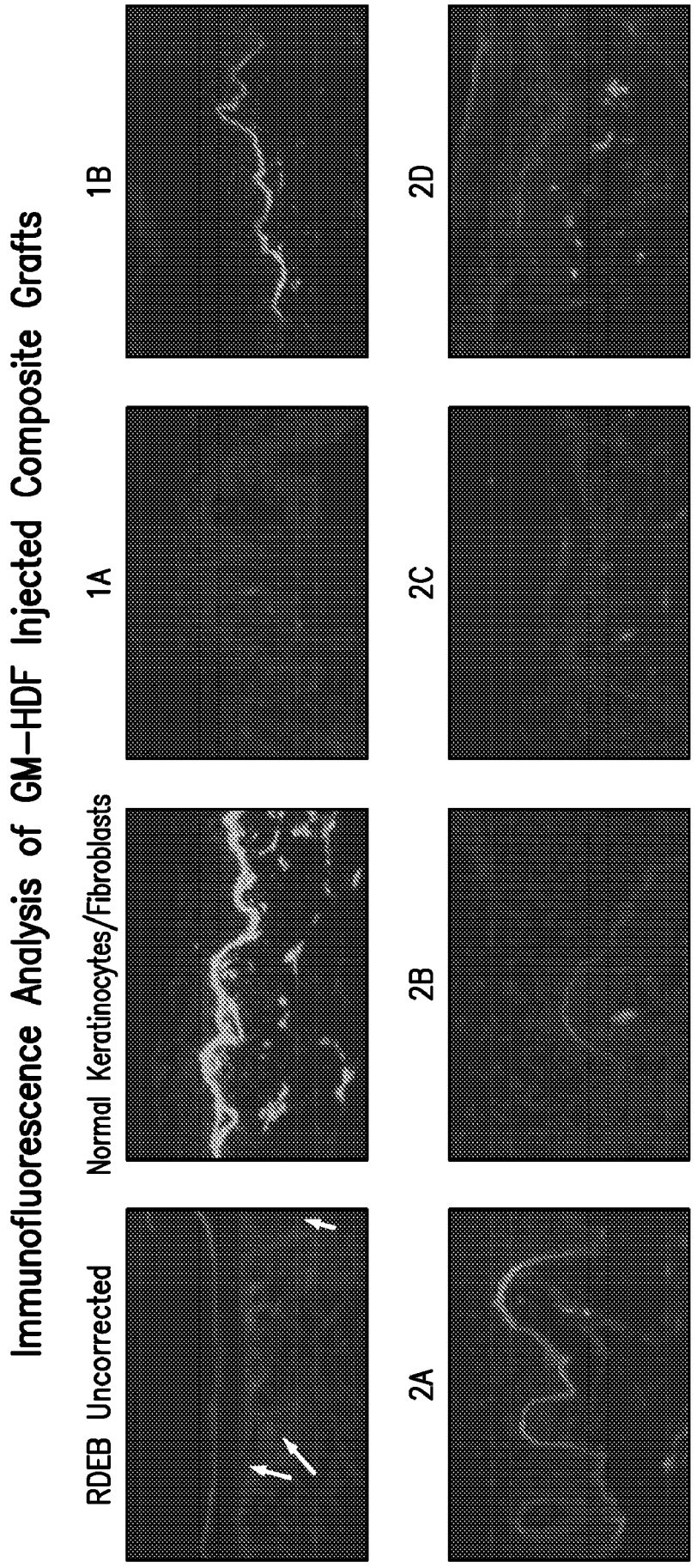

Immunofluorescence Analysis of GM-HDF Injected Composite Grafts
RDEB Uncorrected
Normal Keratinocytes/Fibroblasts
1A
1B
2A
2B
2C
2D

FIG. 26

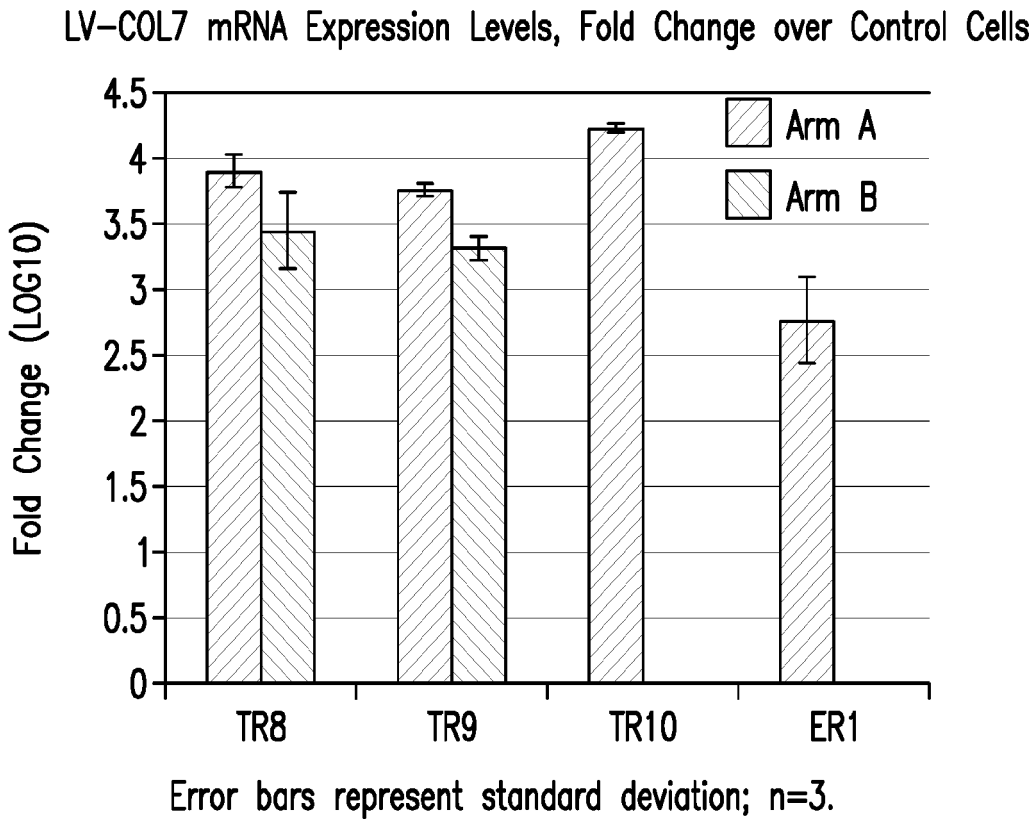

LV-COL7 mRNA Expression Levels, Fold Change over Control Cells
Fold Change (LOG10)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Arm A
Arm B
TR8
TR9
TR10
ER1
Error bars represent standard deviation; n=3.

FIG. 27

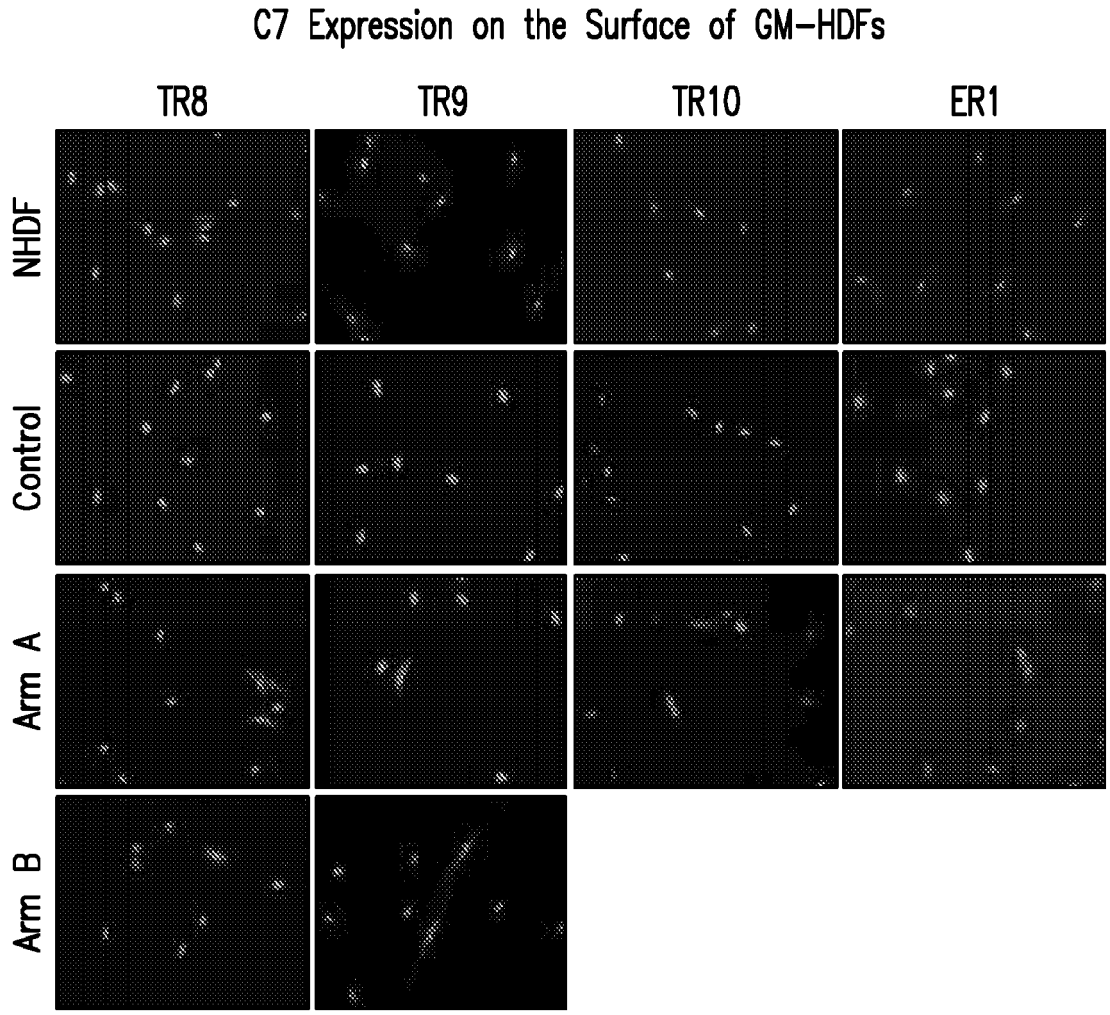

C7 Expression on the Surface of GM-HDFs
TR8
TR9
TR10
ER1
NHDF
Control
Arm A
Arm B

FIG. 28

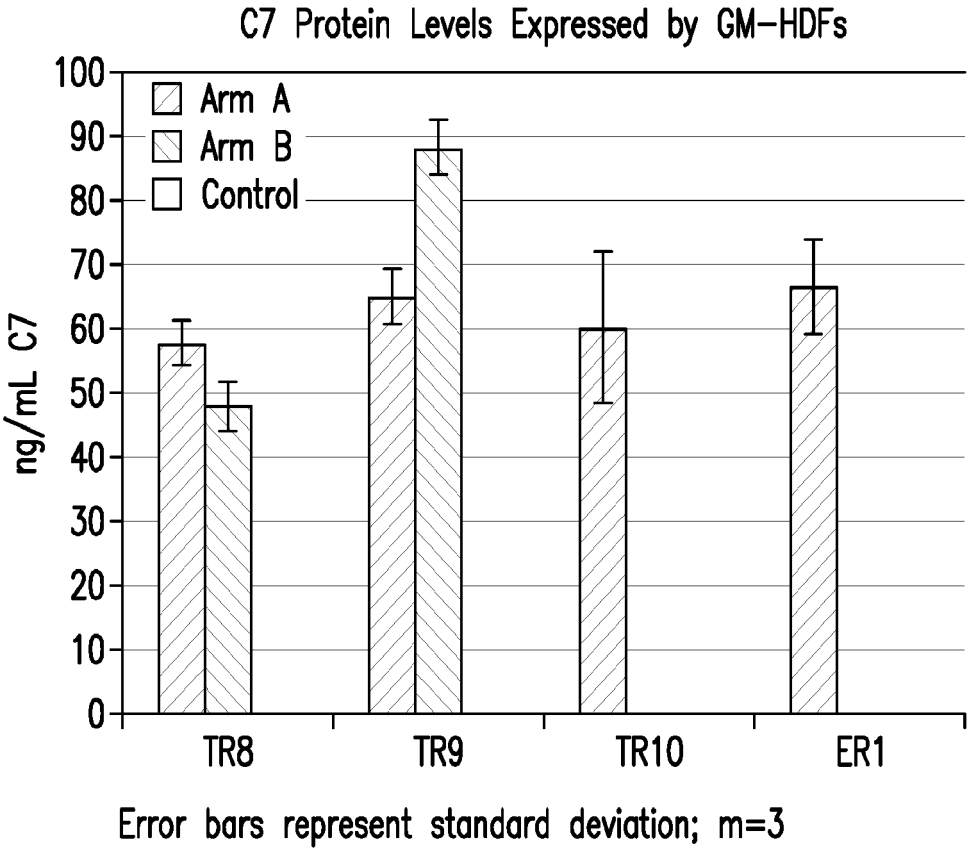

C7 Protein Levels Expressed by GM-HDFs
Arm A
Arm B
Control
ng/mL C7
100
90
80
70
60
50
40
30
20
10
0
TR8
TR9
TR10
ER1
Error bars represent standard deviation; m=3

FIG. 29

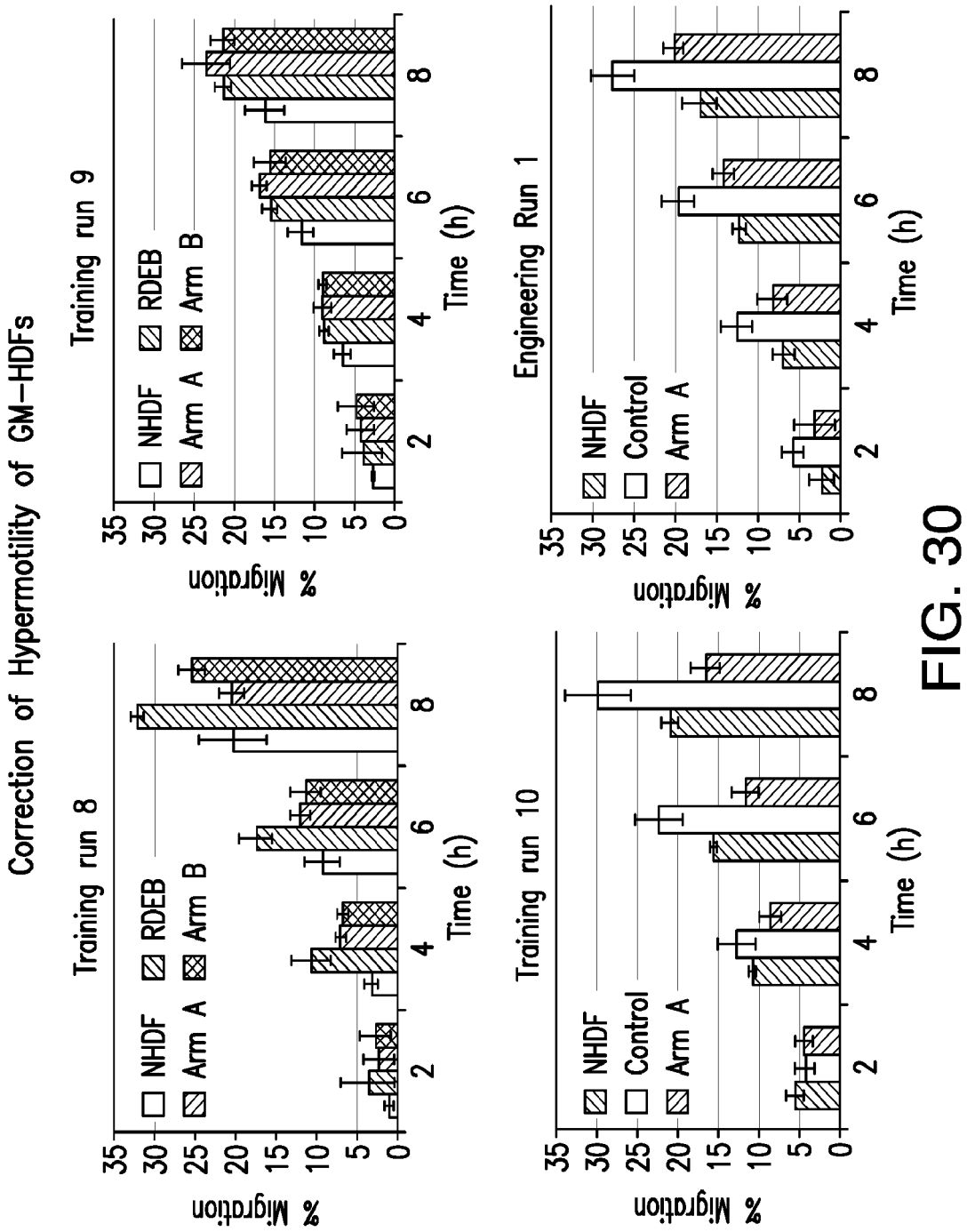

Correction of Hypermotility of GM-HDFs
Training run 8
NHDF
RDEB
Arm A
Arm B
% Migration
Time (h)
Training run 9
Engineering Run 1
Control
Training run 10

FIG. 30

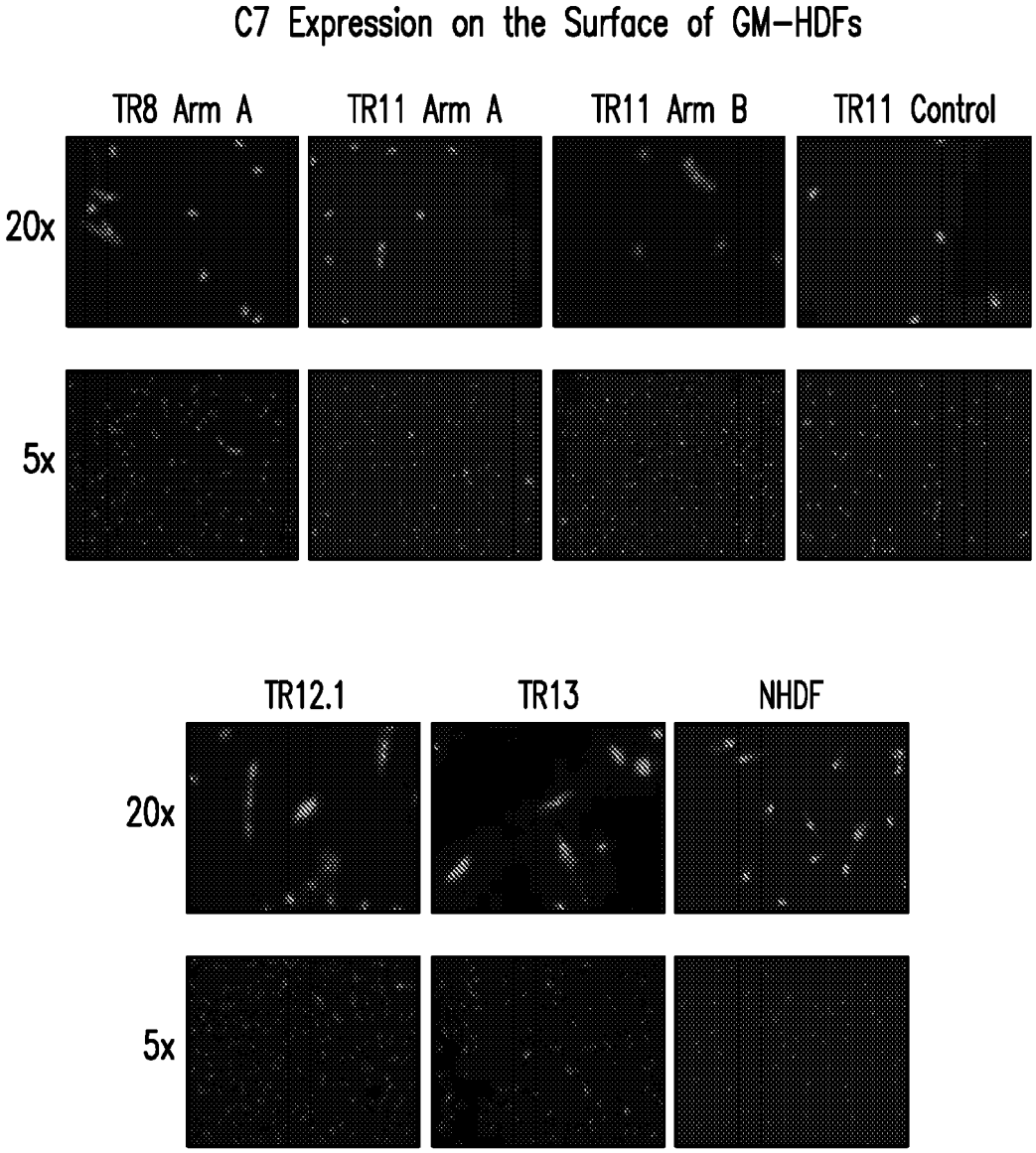

C7 Expression on the Surface of GM-HDFs
TR8 Arm A
TR11 Arm A
TR11 Arm B
TR11 Control
20x
5x
TR12.1
TR13
NHDF
20x
5x

FIG. 31

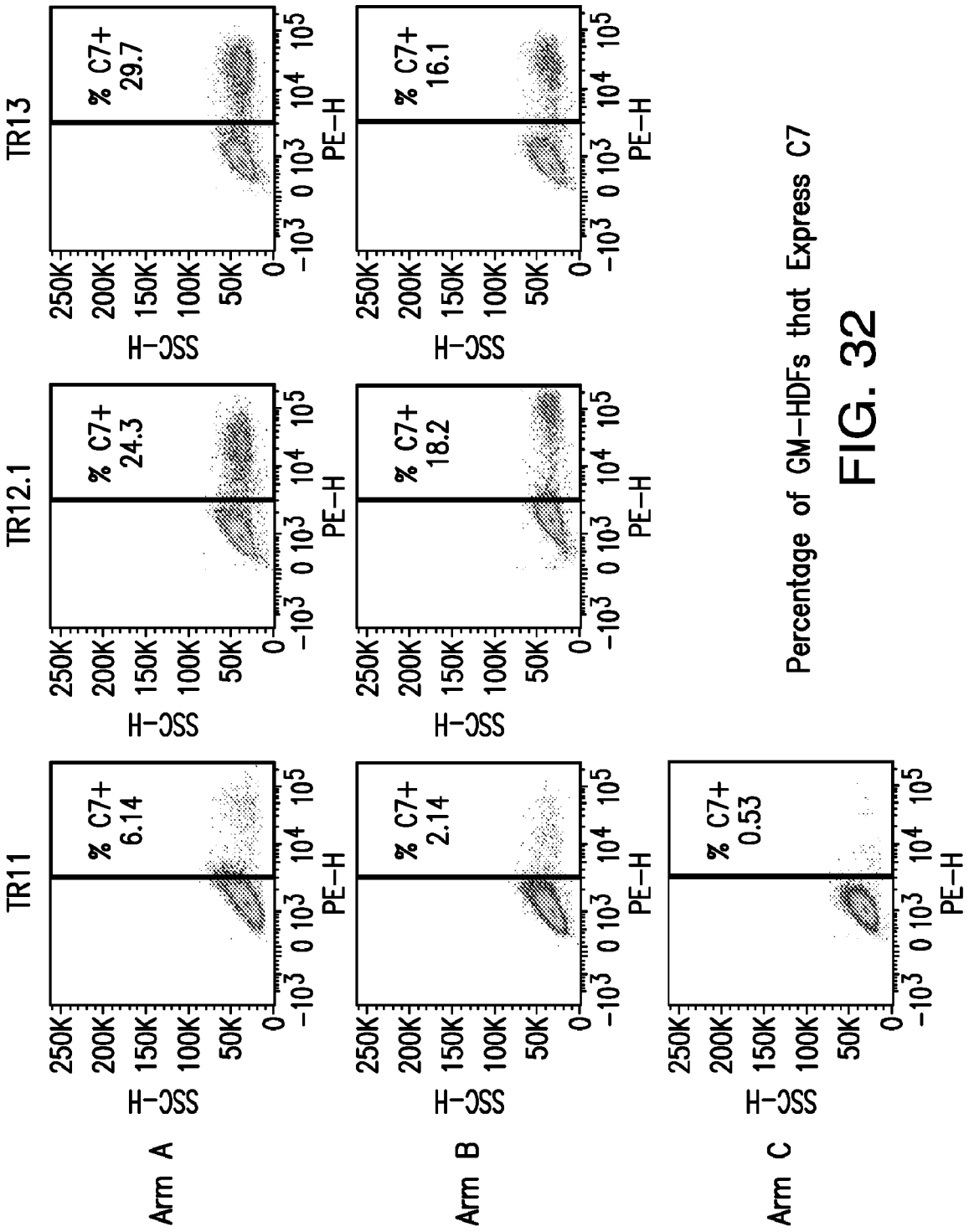

TR11
TR12.1
TR13
Arm A
Arm B
Arm C
% C7+ 6.14
% C7+ 24.3
% C7+ 29.7
% C7+ 2.14
% C7+ 18.2
% C7+ 16.1
% C7+ 0.53
SSC-H
PE-H
250K
200K
150K
100K
50K
0
-10^3 0 10^3 10^4 10^5
Percentage of GM-HDFs that Express C7

FIG. 32

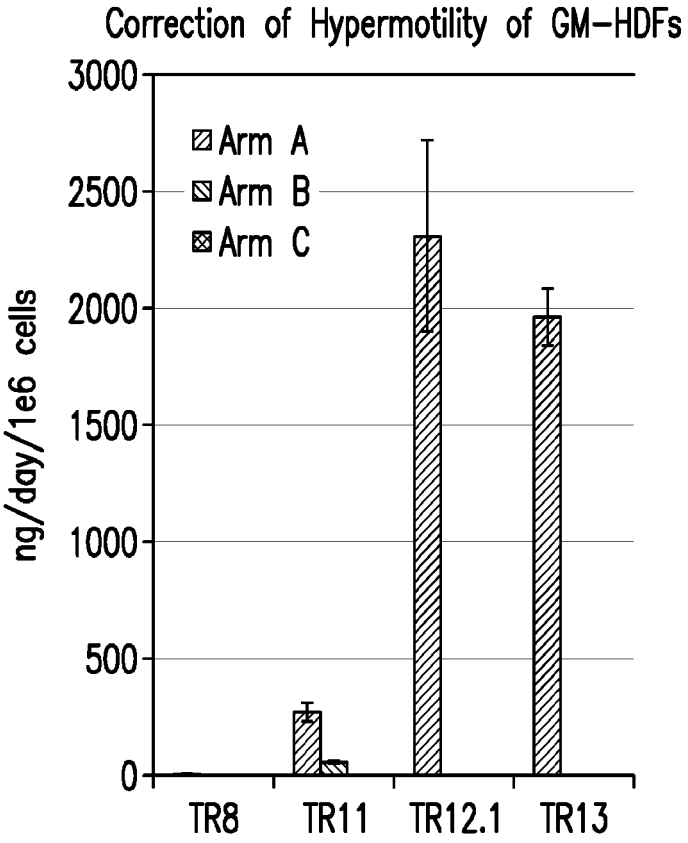

Correction of Hypermotility of GM-HDFs
3000
2500
2000
1500
1000
500
0
ng/day/1e6 cells
Arm A
Arm B
Arm C
TR8
TR11
TR12.1
TR13

FIG. 33

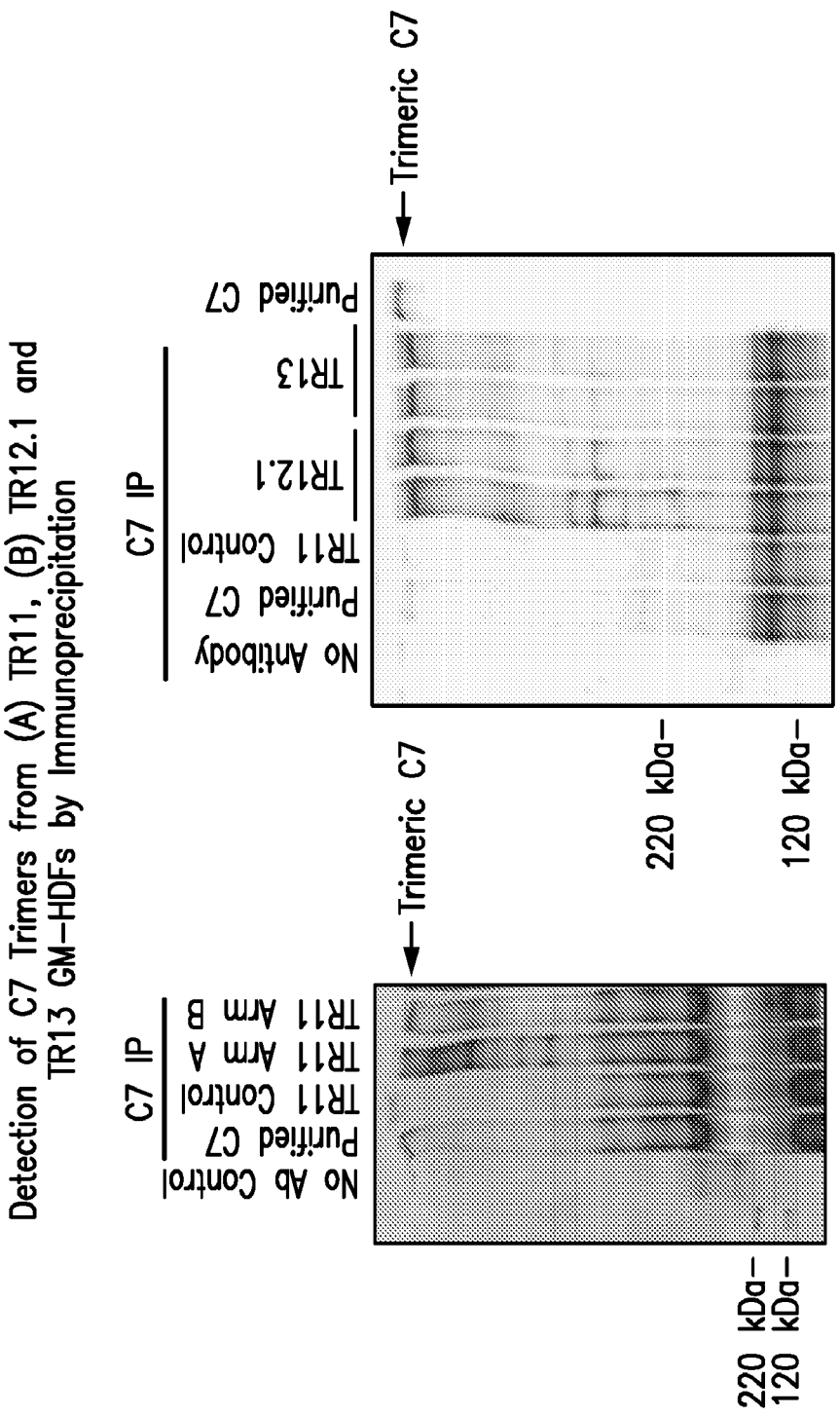

Detection of C7 Trimers from (A) TR11, (B) TR12.1 and TR13 GM-HDFs by Immunoprecipitation
C7 IP
No Ab Control
Purified C7
TR11 Control
TR11 Arm A
TR11 Arm B
Trimeric C7
220 kDa
120 kDa
C7 IP
No Antibody
Purified C7
TR11 Control
TR12.1
TR13
Purified C7
Trimeric C7
220 kDa
120 kDa

FIG. 34

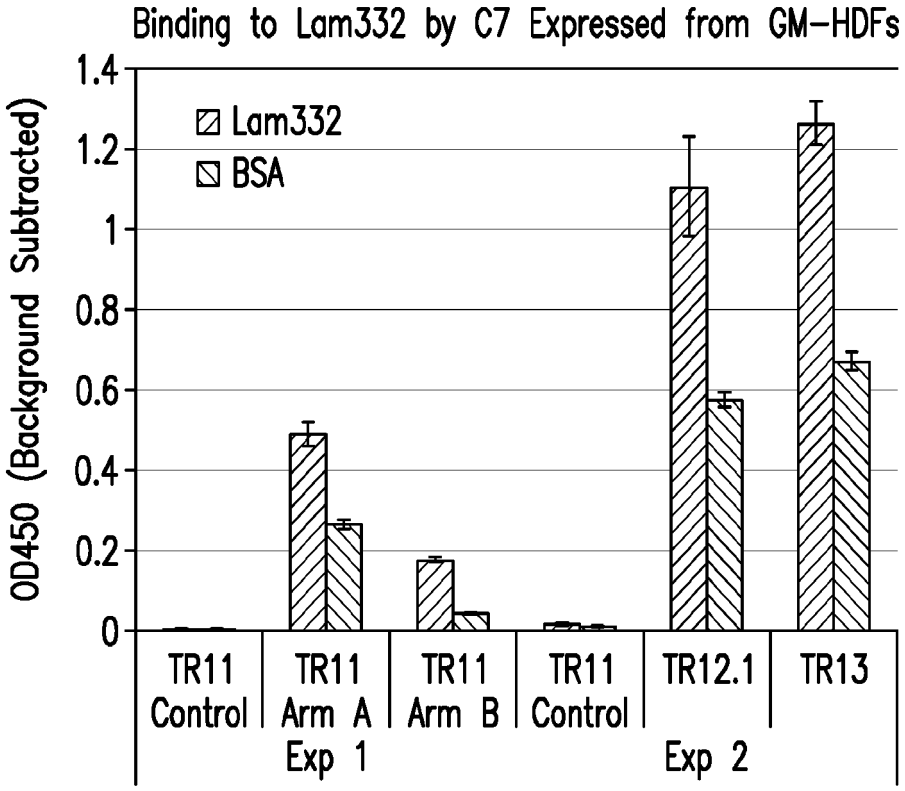

Binding to Lam332 by C7 Expressed from GM-HDFs
OD450 (Background Subtracted)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Lam332
BSA
TR11 Control
TR11 Arm A
TR11 Arm B
TR11 Control
TR12.1
TR13
Exp 1
Exp 2

FIG. 35

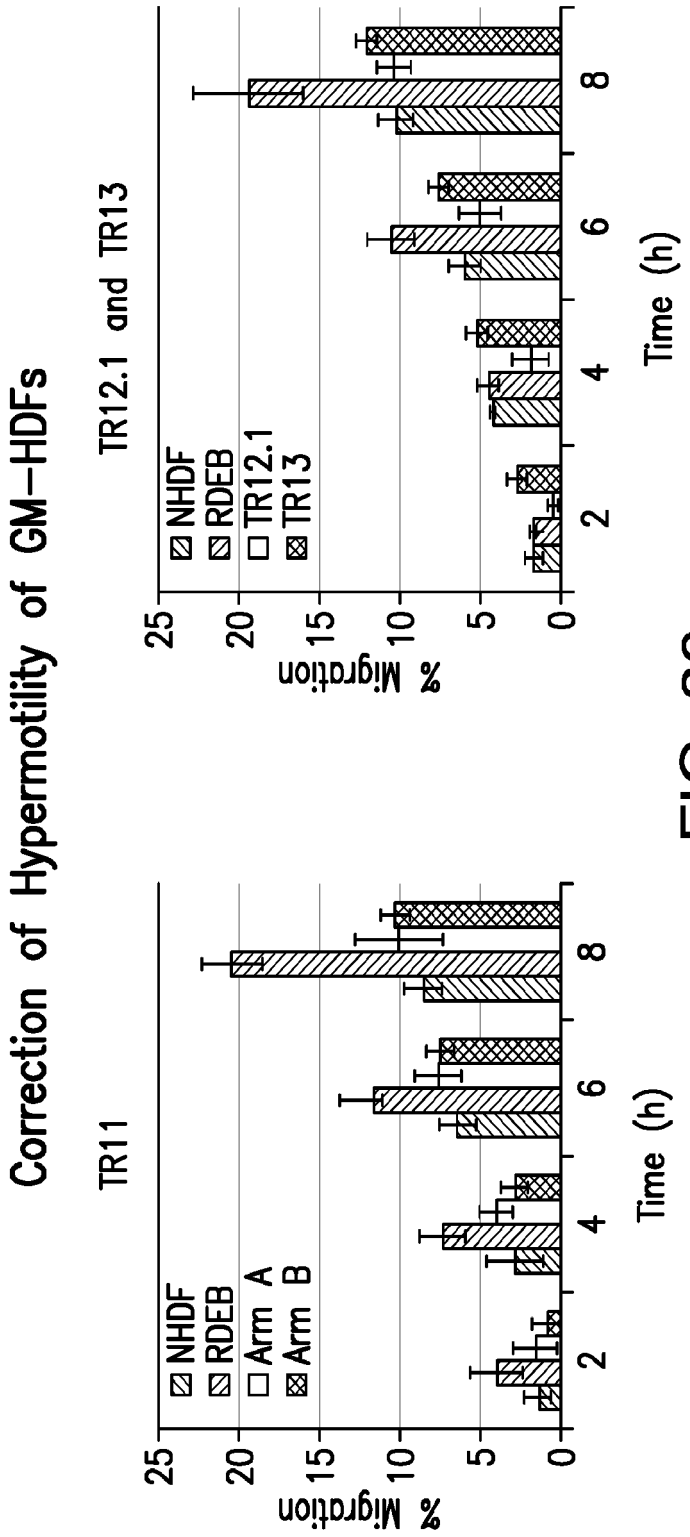

Correction of Hypermotility of GM-HDFs
TR11
NHDF
RDEB
Arm A
Arm B
% Migration
Time (h)
TR12.1 and TR13
NHDF
RDEB
TR12.1
TR13
% Migration
Time (h)

FIG. 36