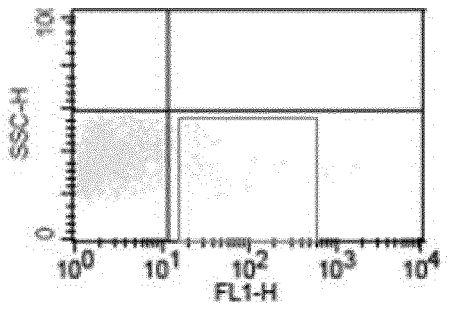
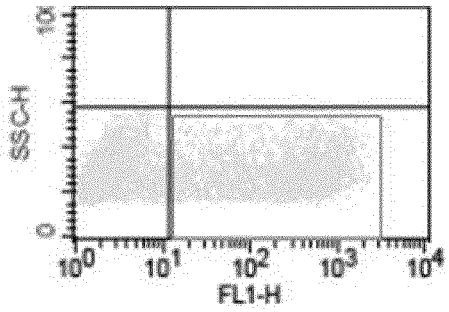
Figure 1

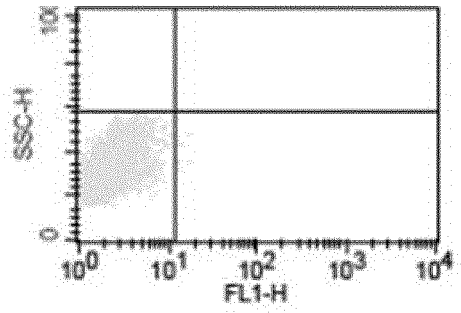
a)



b)



c)



d)

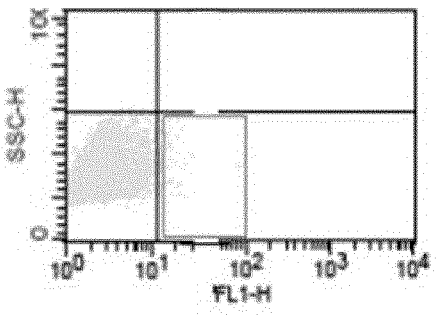
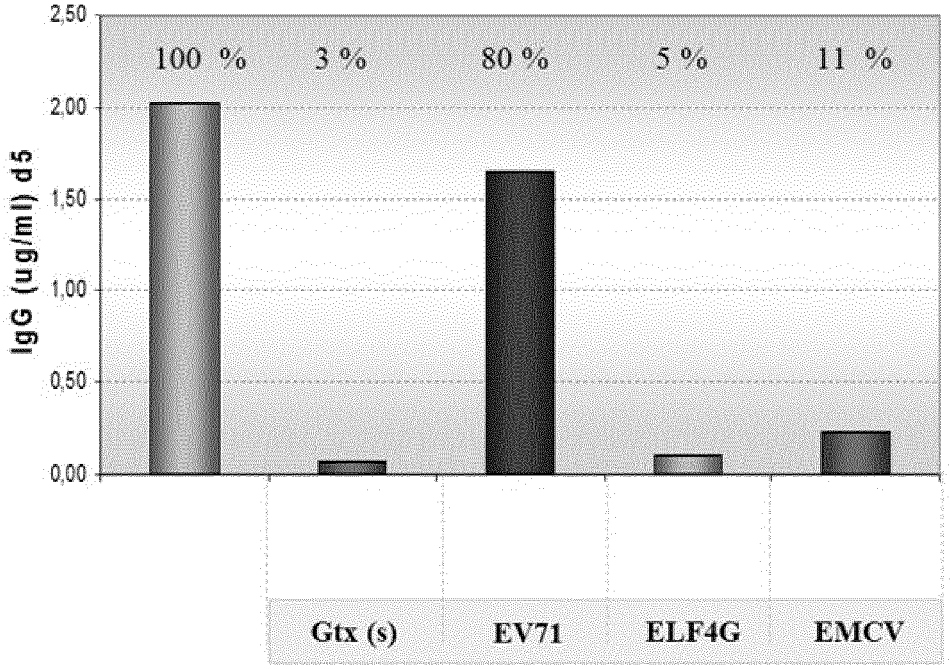


Figure 2


2,50
2,00
1,50
1,00
0,50
0,00
IgG (ug/ml) d5
100 %
3 %
80 %
5 %
11 %
Gtx (s)
EV71
ELF4G
EMCV

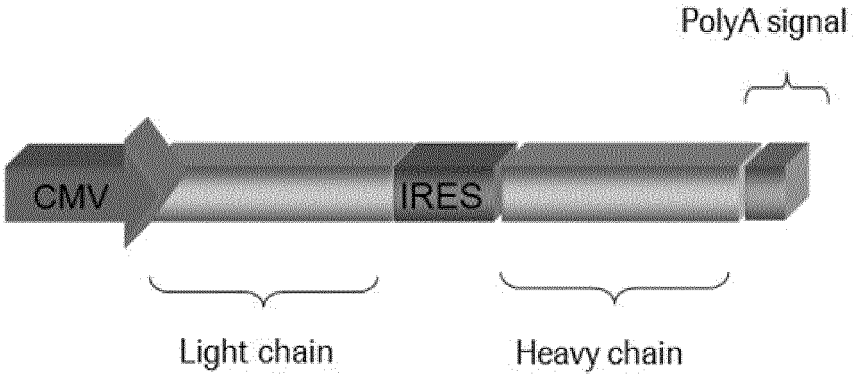
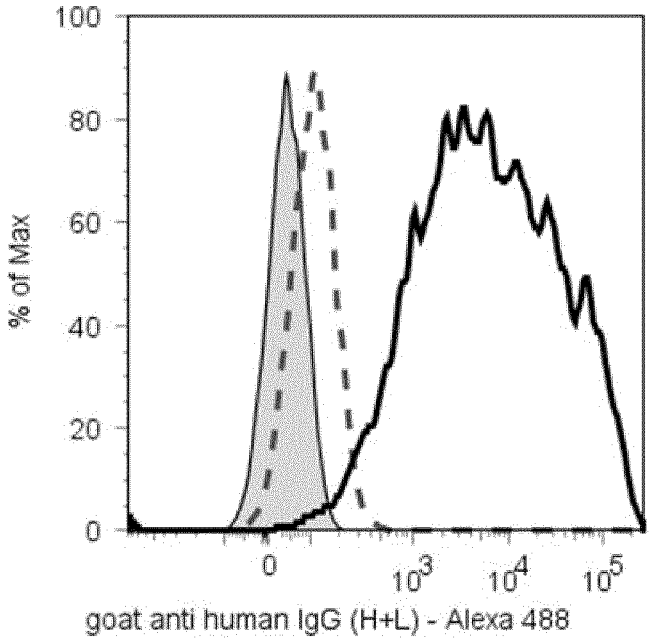

PolyA signal
CMV
IRES
Light chain
Heavy chain

Figure 3

a)


100
80
60
40
20
0
% of Max
0
10^3
10^4
10^5
goat anti human IgG (H+L) - Alexa 488

b)

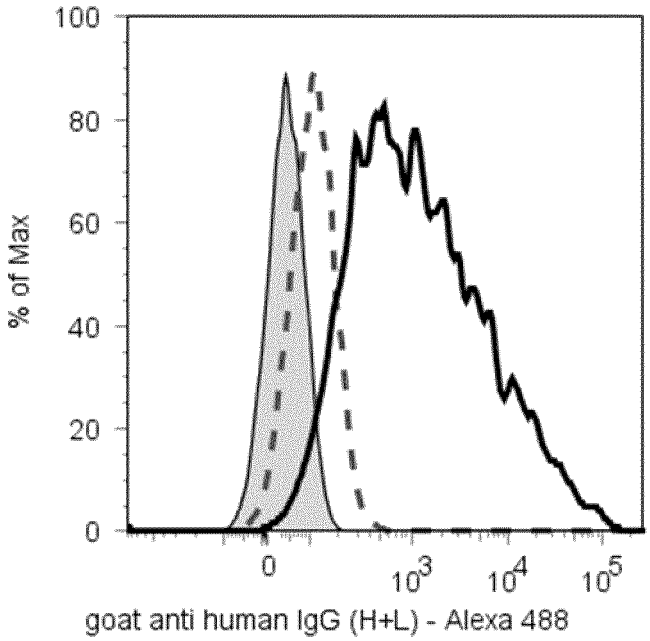
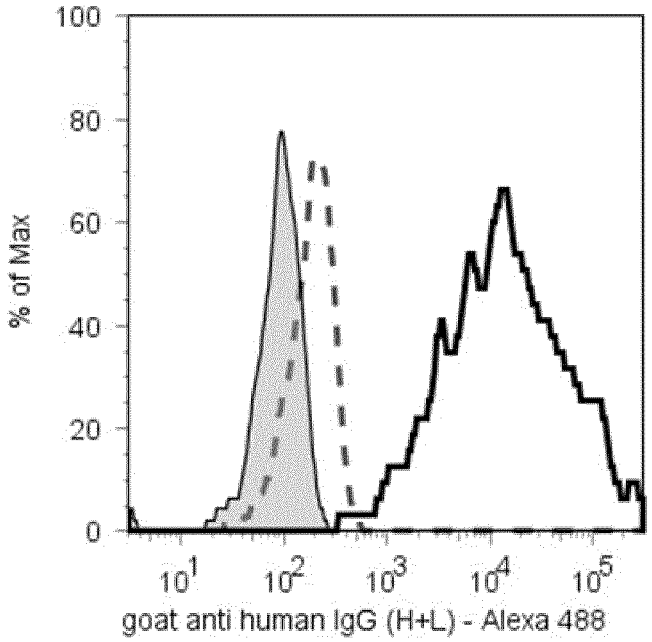

100
80
60
40
20
0
% of Max
0
10^3
10^4
10^5
goat anti human IgG (H+L) - Alexa 488

Figure 4

a)


100
80
60
40
20
0
% of Max
10^1
10^2
10^3
10^4
10^5
goat anti human IgG (H+L) - Alexa 488

b)

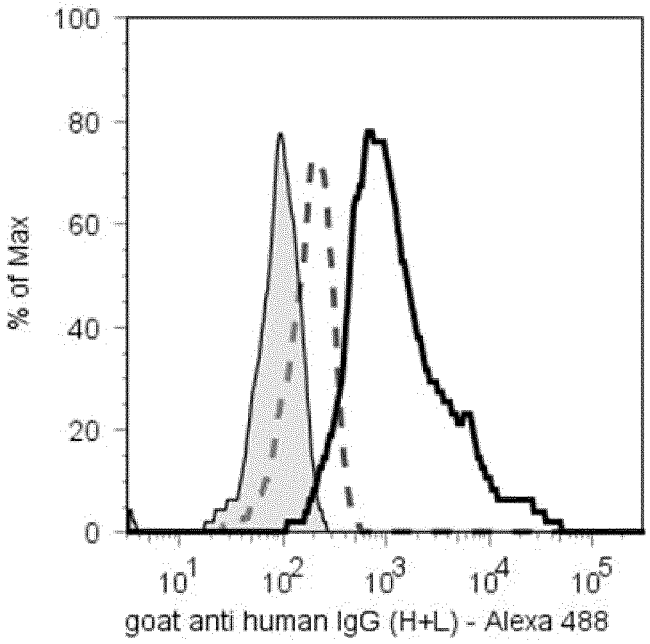

100
80
60
40
20
0
% of Max
10^1
10^2
10^3
10^4
10^5
goat anti human IgG (H+L) - Alexa 488

Figure 5

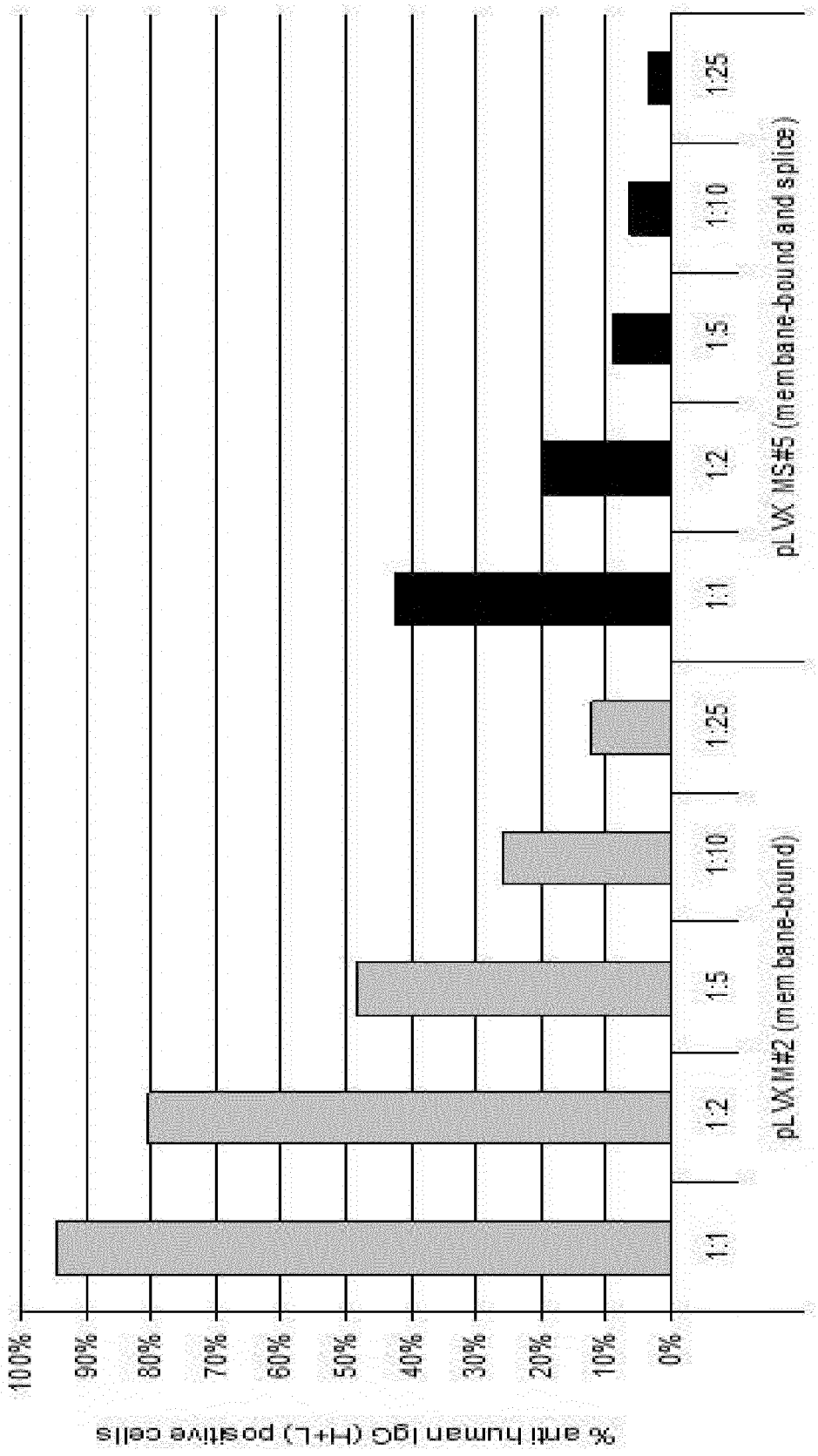
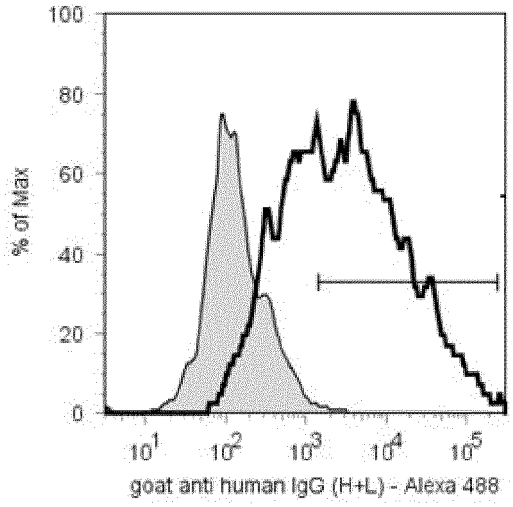
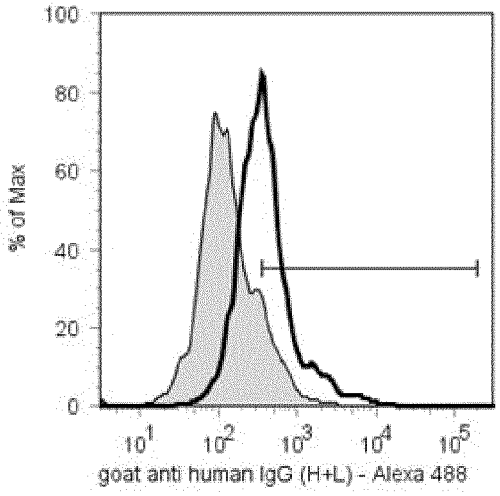

% anti human IgG (H+L) positive cells
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1:1
1:2
1:5
1:10
1:25
pLWM#2 (membane-bound)
1:1
1:2
1:5
1:10
1:25
pLWMS#5 (membane-bound and splice)

Figure 6

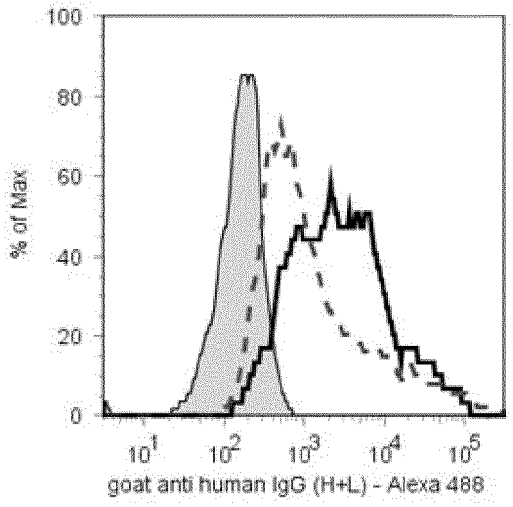
a)



c)



b)



d)

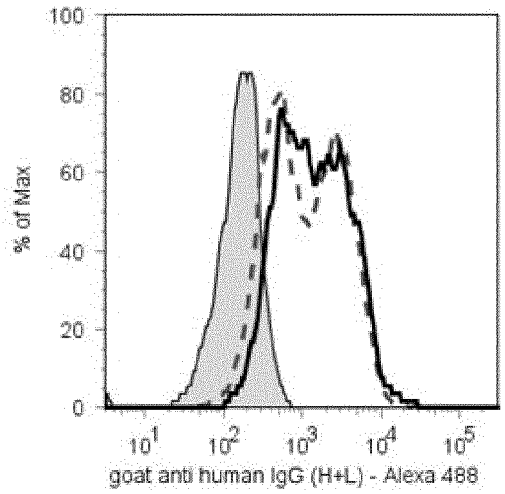


Figure 7

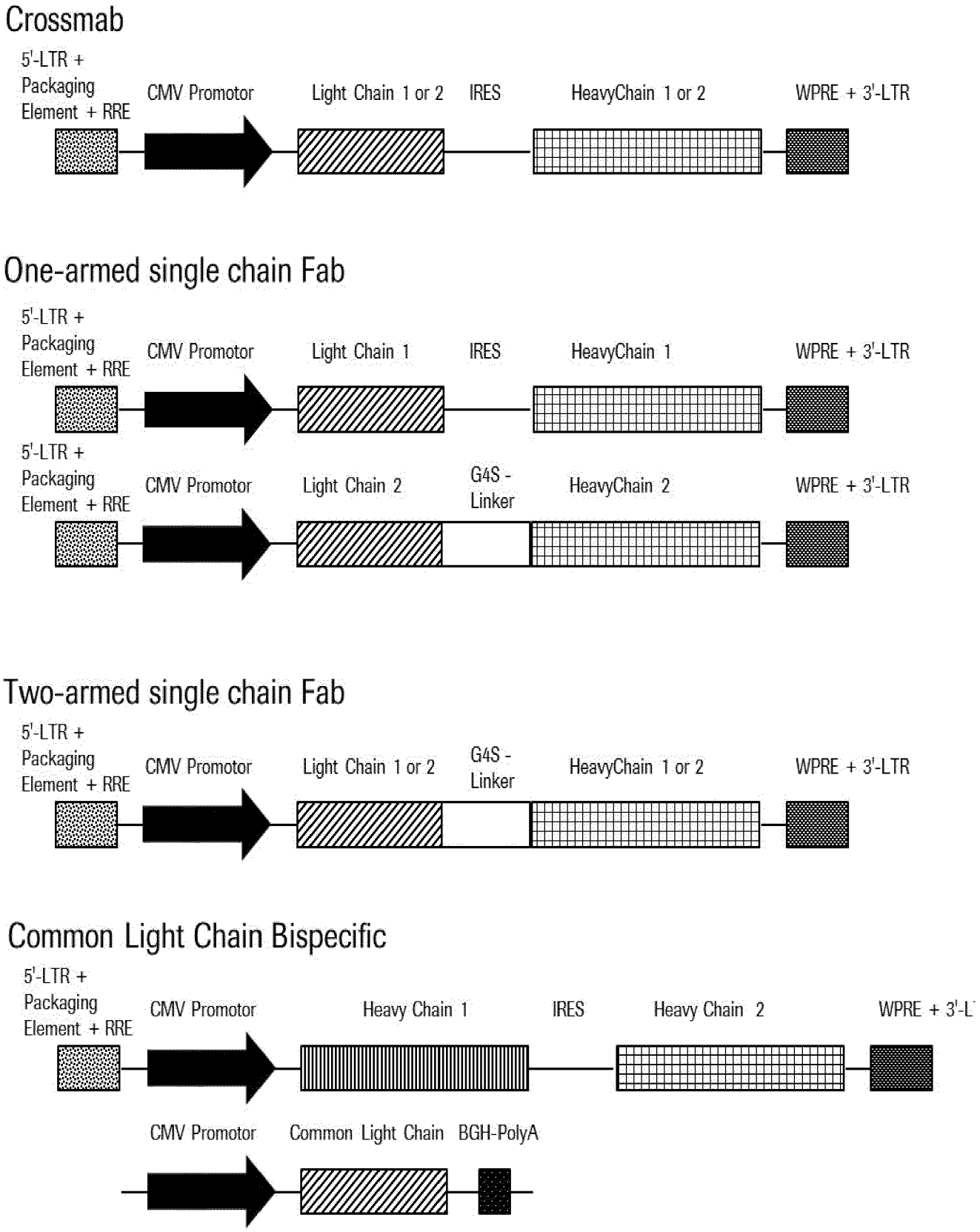

Crossmab
5'-LTR + Packaging Element + RRE
CMV Promotor
Light Chain 1 or 2
IRES
HeavyChain 1 or 2
WPRE + 3'-LTR
One-armed single chain Fab
5'-LTR + Packaging Element + RRE
CMV Promotor
Light Chain 1
IRES
HeavyChain 1
WPRE + 3'-LTR
5'-LTR + Packaging Element + RRE
CMV Promotor
Light Chain 2
G4S - Linker
HeavyChain 2
WPRE + 3'-LTR
Two-armed single chain Fab
5'-LTR + Packaging Element + RRE
CMV Promotor
Light Chain 1 or 2
G4S - Linker
HeavyChain 1 or 2
WPRE + 3'-LTR
Common Light Chain Bispecific
5'-LTR + Packaging Element + RRE
CMV Promotor
Heavy Chain 1
IRES
Heavy Chain 2
WPRE + 3'-L
CMV Promotor
Common Light Chain
BGH-PolyA

Figure 7 (continued)

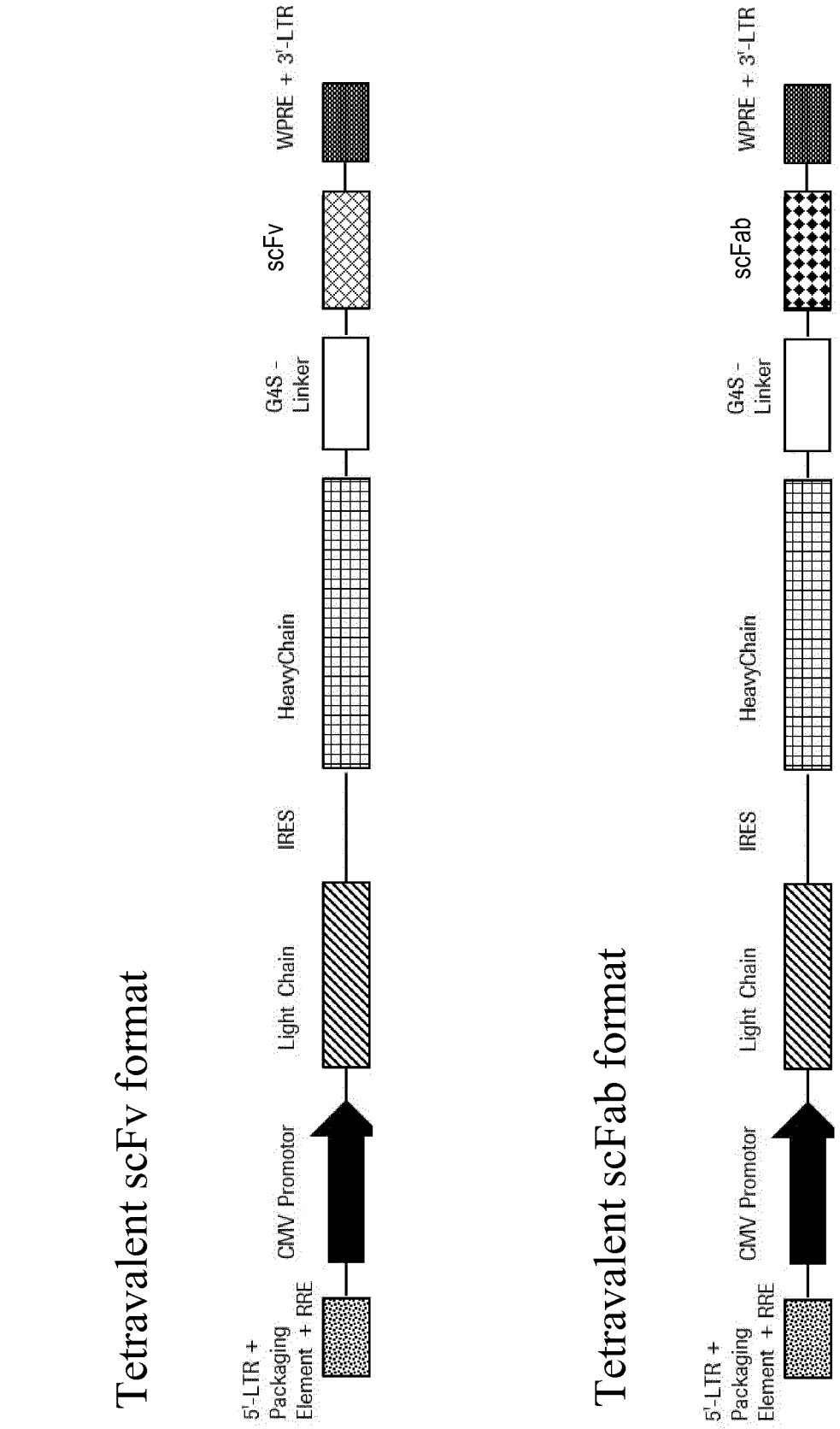


Figure 8

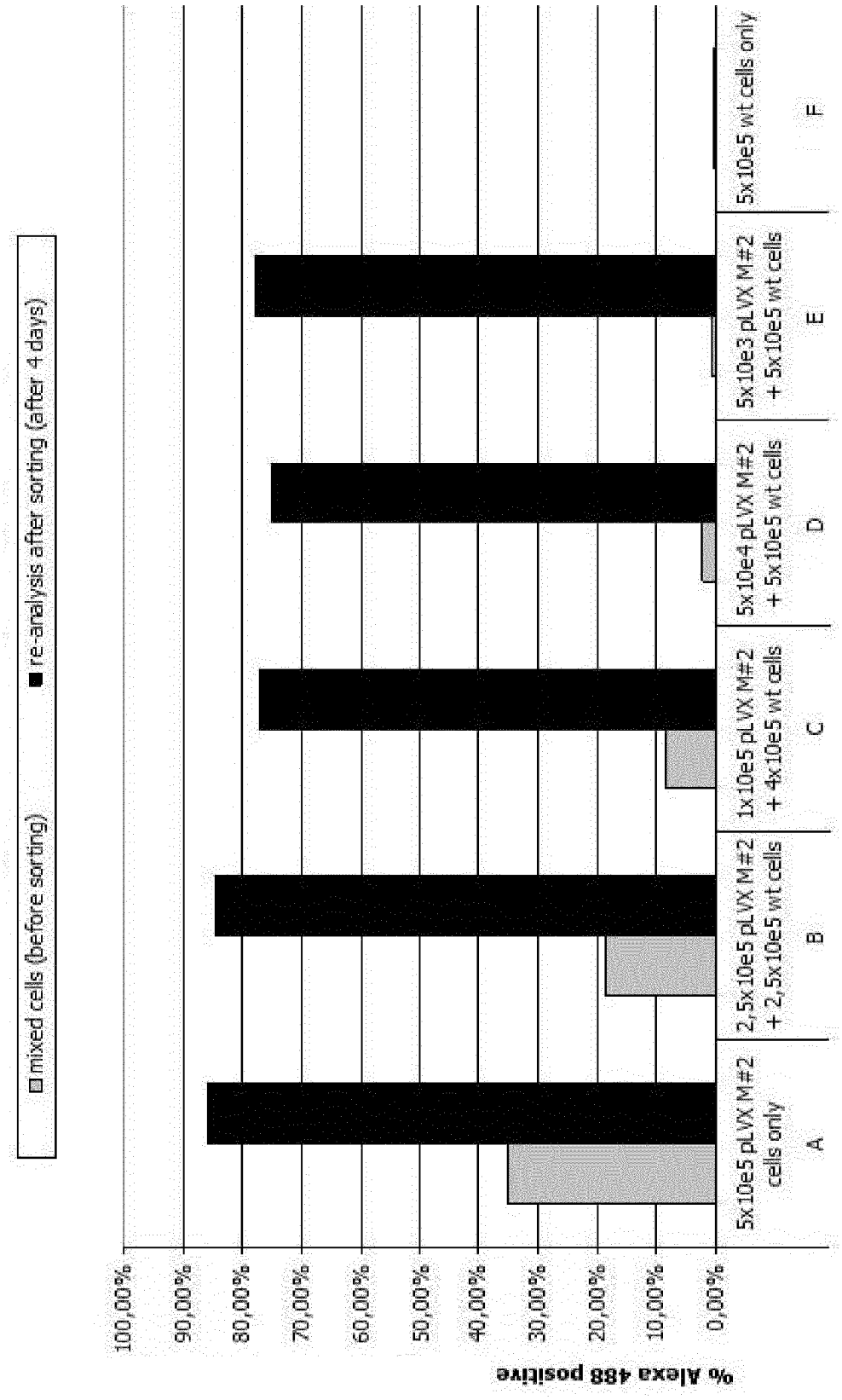

mixed cells (before sorting)
re-analysis after sorting (after 4 days)
% Alexa 488 positive
100,00%
90,00%
80,00%
70,00%
60,00%
50,00%
40,00%
30,00%
20,00%
10,00%
0,00%
5x10e5 pLVX M#2 cells only
A
2,5x10e5 pLVX M#2 + 2,5x10e5 wt cells
B
1x10e5 pLVX M#2 + 4x10e5 wt cells
C
5x10e4 pLVX M#2 + 5x10e5 wt cells
D
5x10e3 pLVX M#2 + 5x10e5 wt cells
E
5x10e5 wt cells only
F

Figure 9

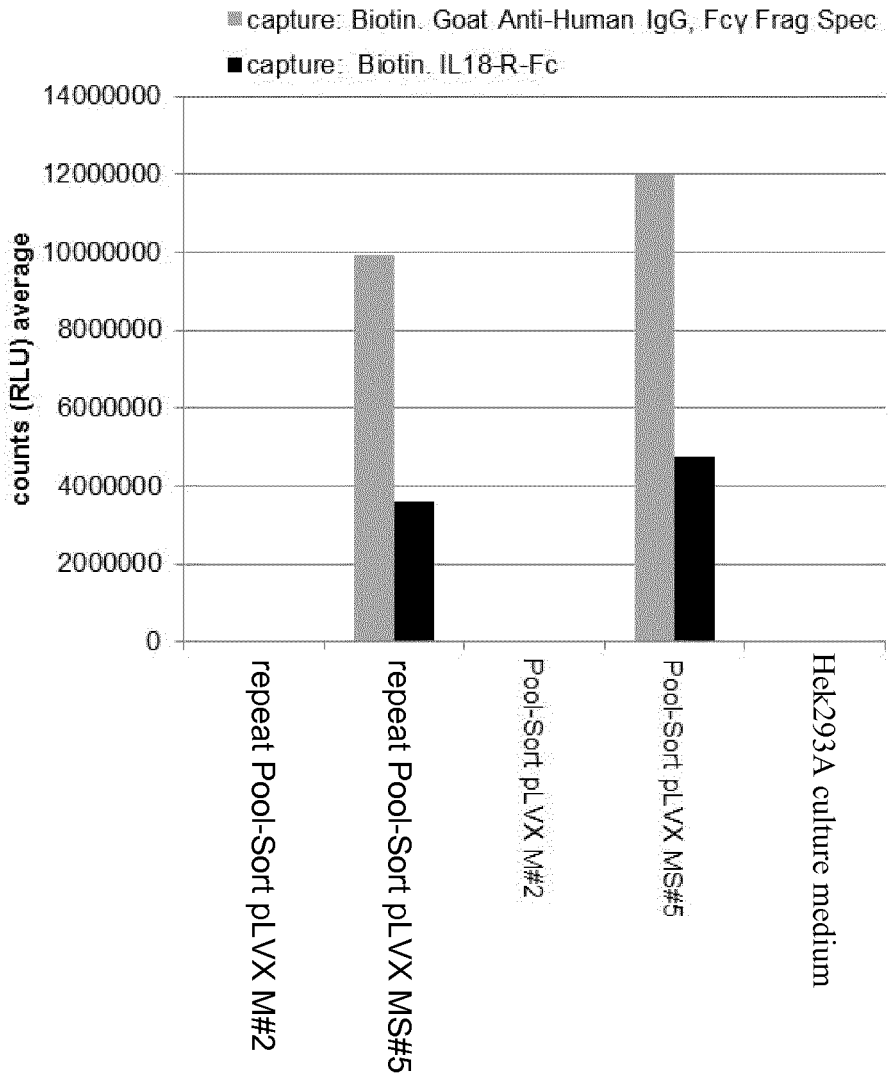

capture: Biotin. Goat Anti-Human IgG, Fcγ Frag Spec
capture: Biotin. IL18-R-Fc
counts (RLU) average
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
repeat Pool-Sort pLVX M#2
repeat Pool-Sort pLVX MS#5
Pool-Sort pLVX M#2
Pool-Sort pLVX MS#5
Hek293A culture medium

Figure 10

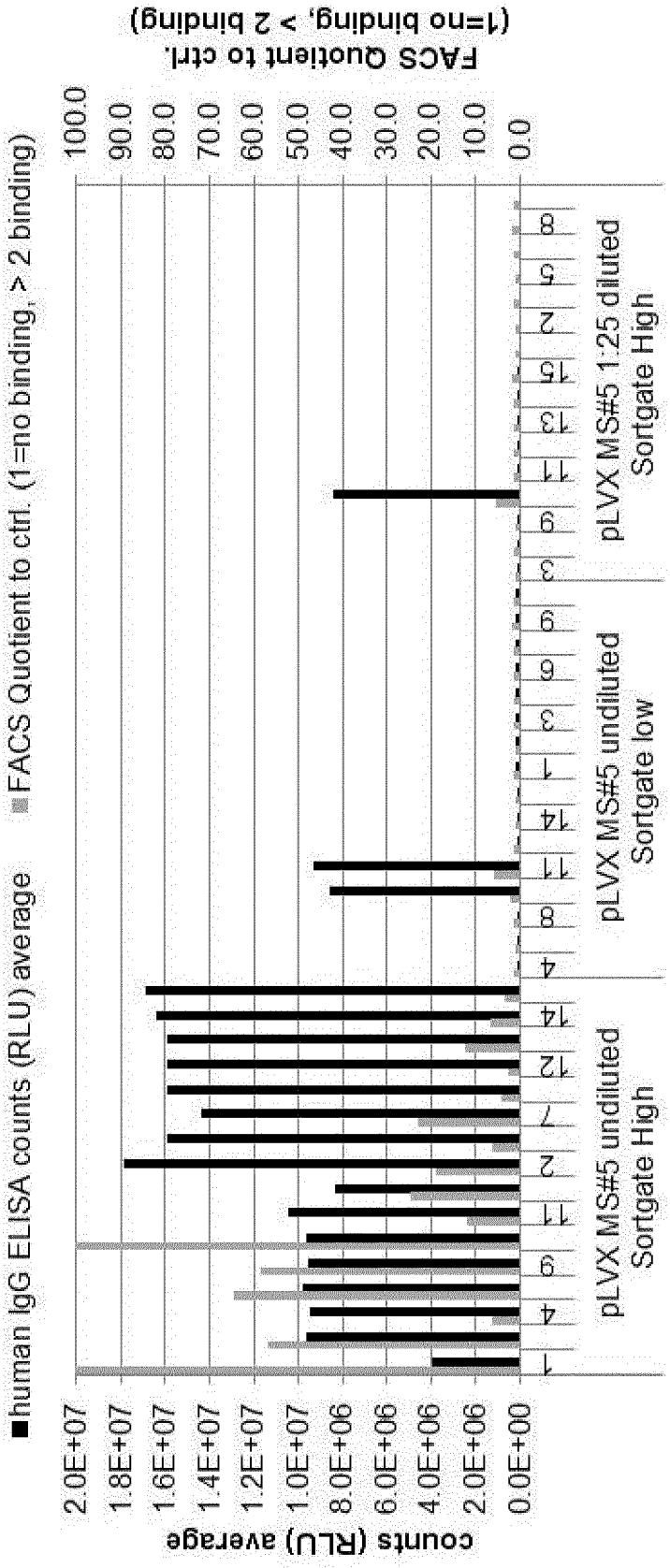

FACS Quotient to ctrl.
(1=no binding, > 2 binding)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
counts (RLU) average
2.0E+07
1.8E+07
1.6E+07
1.4E+07
1.2E+07
1.0E+07
8.0E+06
6.0E+06
4.0E+06
2.0E+06
0.0E+00
human IgG ELISA counts (RLU) average
FACS Quotient to ctrl. (1=no binding, > 2 binding)
pLVX MS#5 undiluted Sortgate High
pLVX MS#5 undiluted Sortgate low
pLVX MS#5 1:25 diluted Sortgate High

Figure 11

(A)

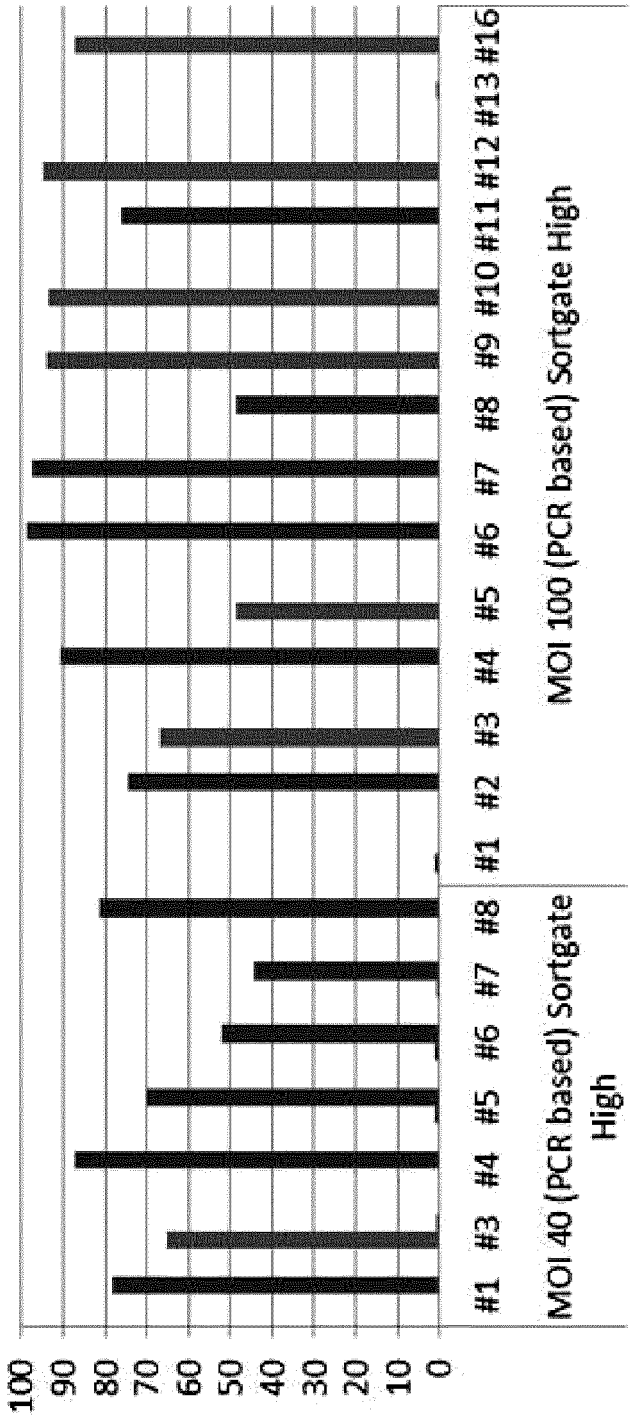

100
90
80
70
60
50
40
30
20
10
0
#1 #3 #4 #5 #6 #7 #8
MOI 40 (PCR based) Sortgate High
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #16
MOI 100 (PCR based) Sortgate High

Figure 11

(A) continued

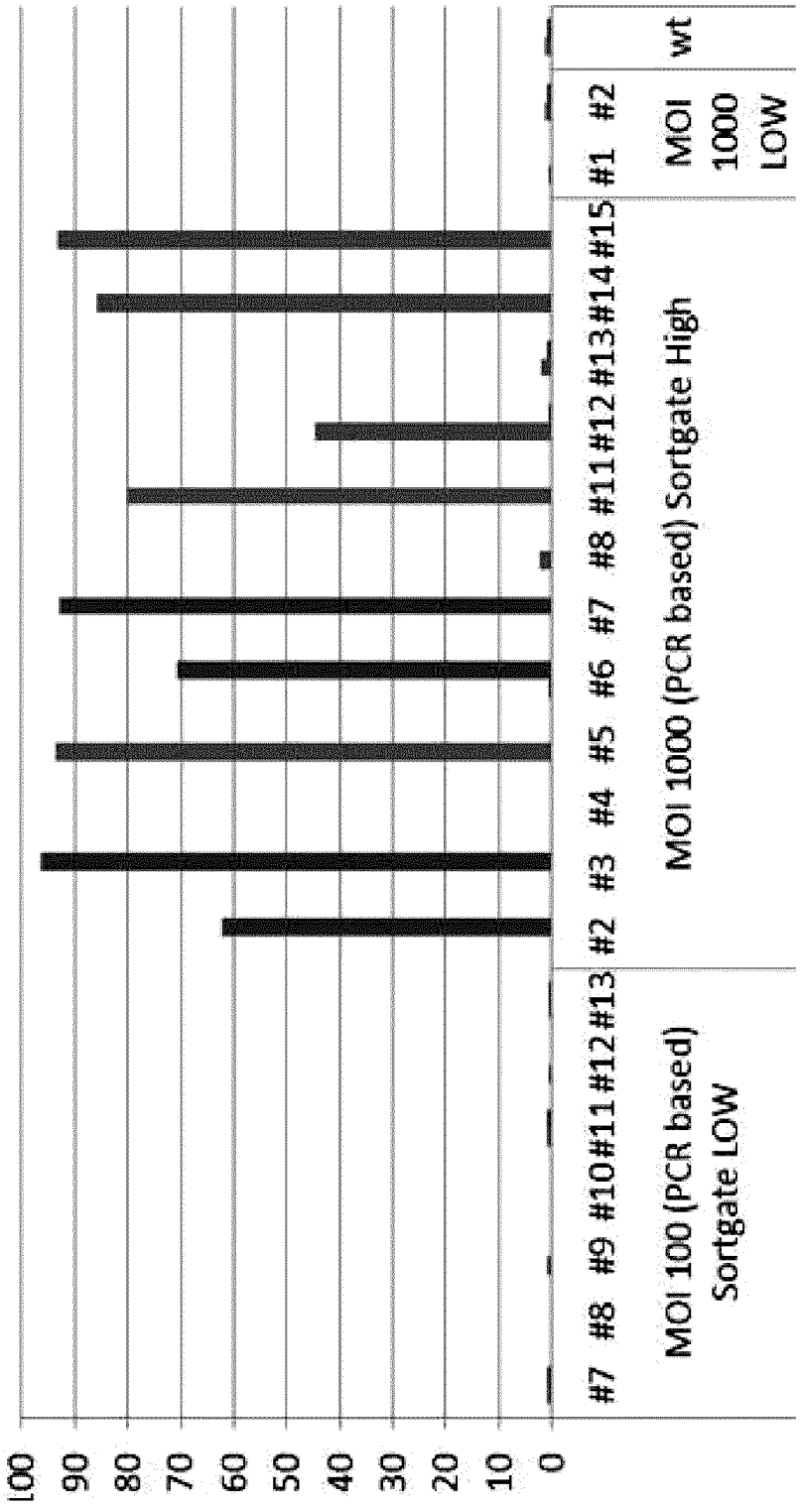


Figure 11

(B)

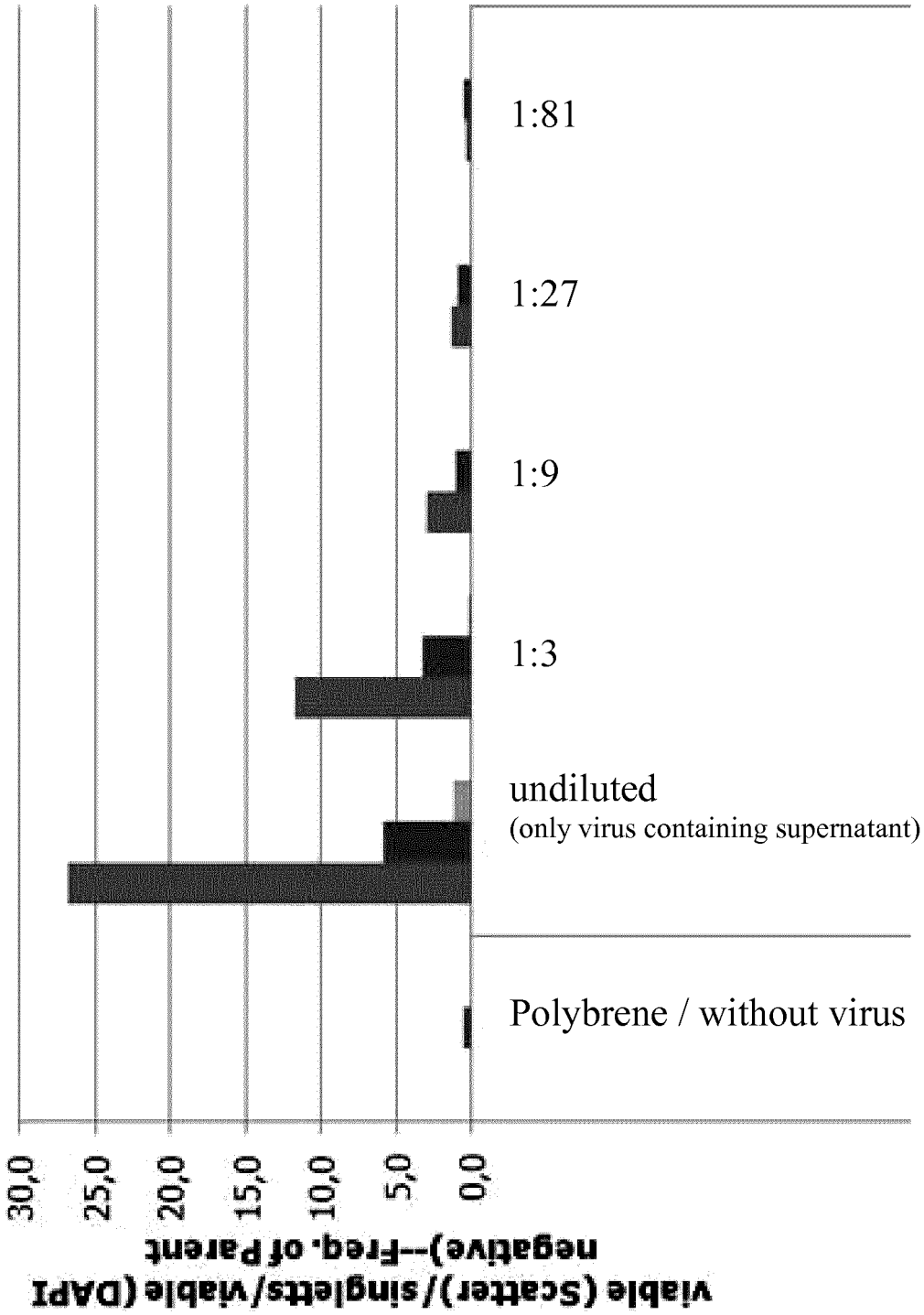

1:81
1:27
1:9
1:3
undiluted
(only virus containing supernatant)
Polybrene / without virus
30,0
25,0
20,0
15,0
10,0
5,0
0,0
viable (Scatter)/singletts/viable (DAPI negative)--Freq. of Parent

Figure 12

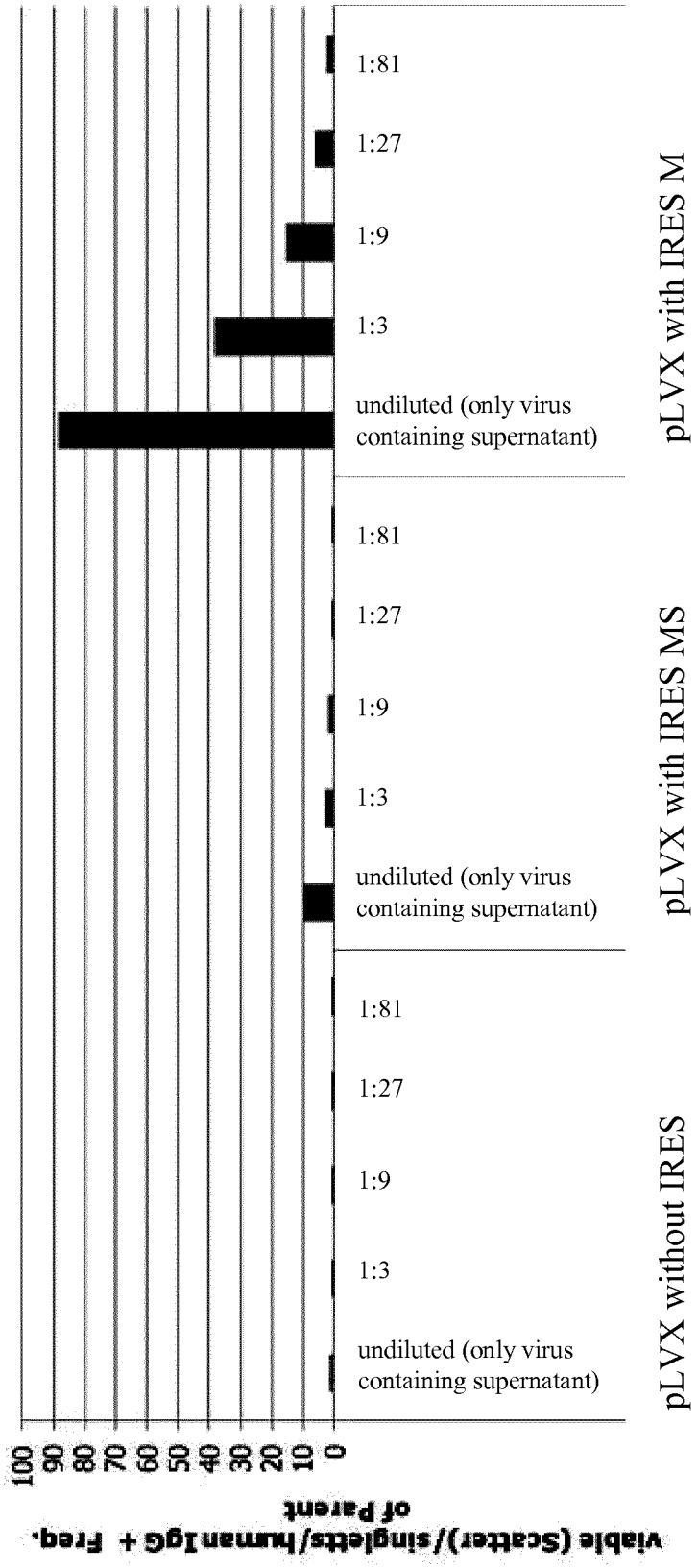

1:81
1:27
1:9
1:3
undiluted (only virus containing supernatant)
pLVX with IRES M
1:81
1:27
1:9
1:3
undiluted (only virus containing supernatant)
pLVX with IRES MS
1:81
1:27
1:9
1:3
undiluted (only virus containing supernatant)
pLVX without IRES
100 90 80 70 60 50 40 30 20 10 0
viable (Scatter)/singlette/human IgG + Freq. of Parent

Figure 13

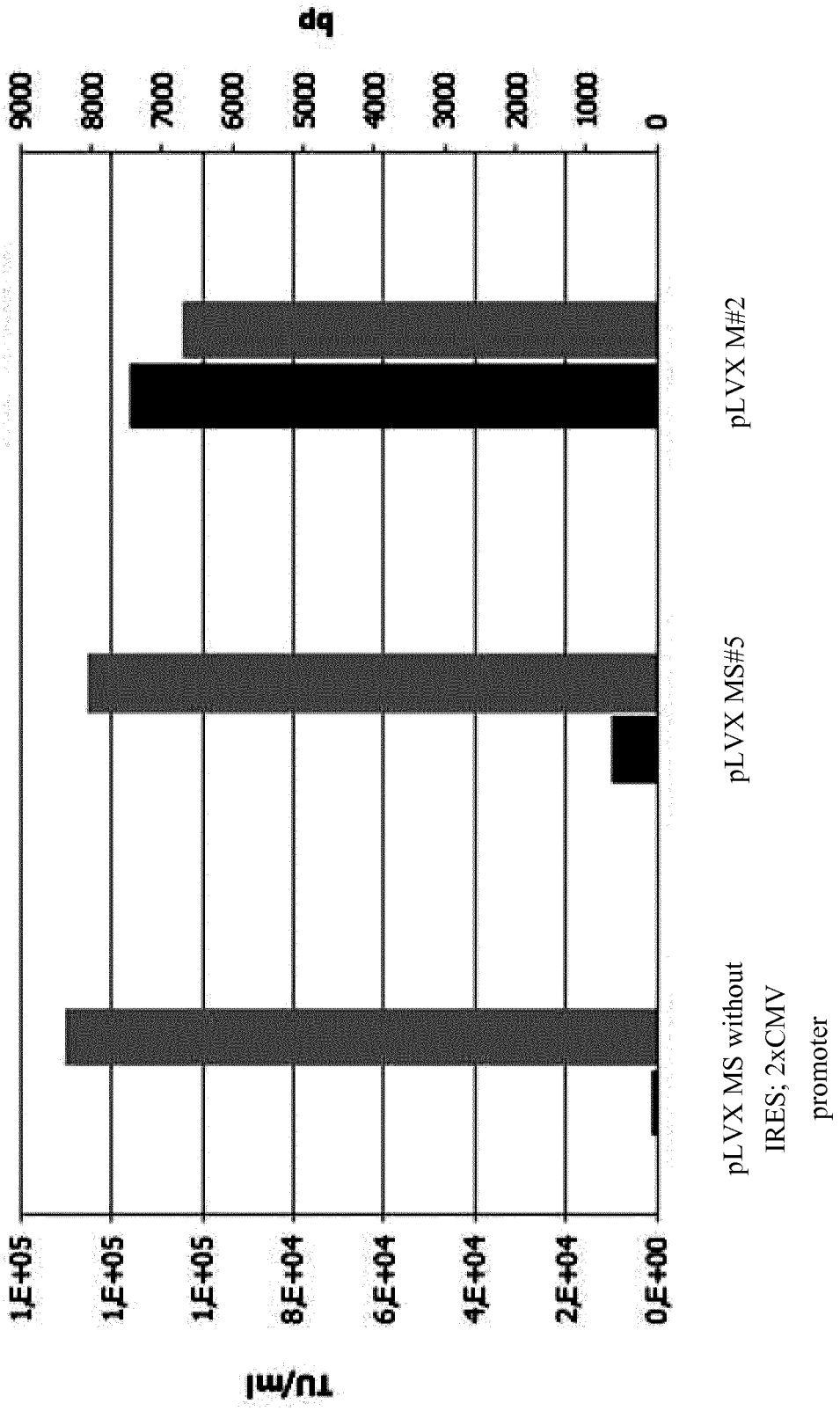

bp
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
TU/ml
1E+05
1E+05
1E+05
8E+04
6E+04
4E+04
2E+04
0E+00
pLVX MS without IRES; 2xCMV promoter
pLVX MS#5
pLVX M#2

Figure 14

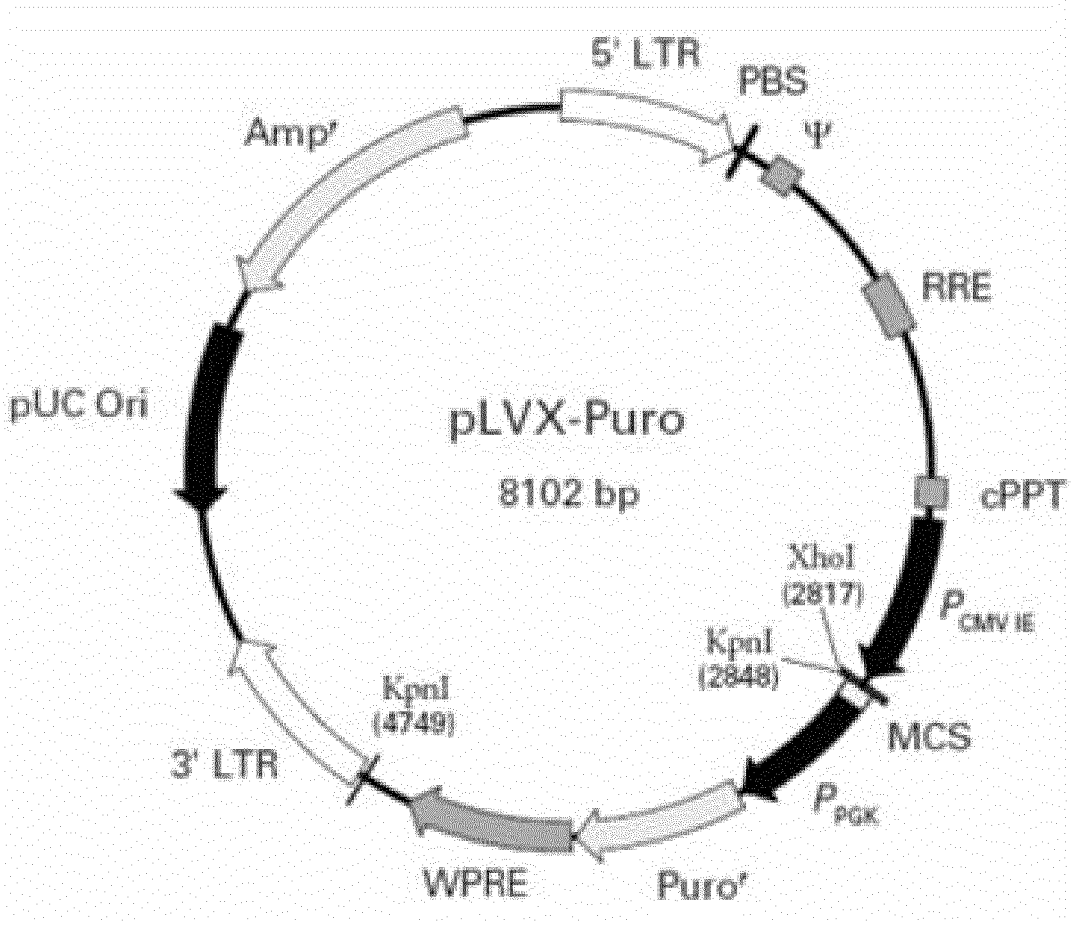

5' LTR
PBS
Ψ
RRE
cPPT
$P_{CMV\ IE}$
MCS
P_{PGK}
Puro'
WPRE
3' LTR
pUC Ori
Amp'
pLVX-Puro
8102 bp
XhoI
(2817)
KpnI
(2848)
KpnI
(4749)

Figure 15

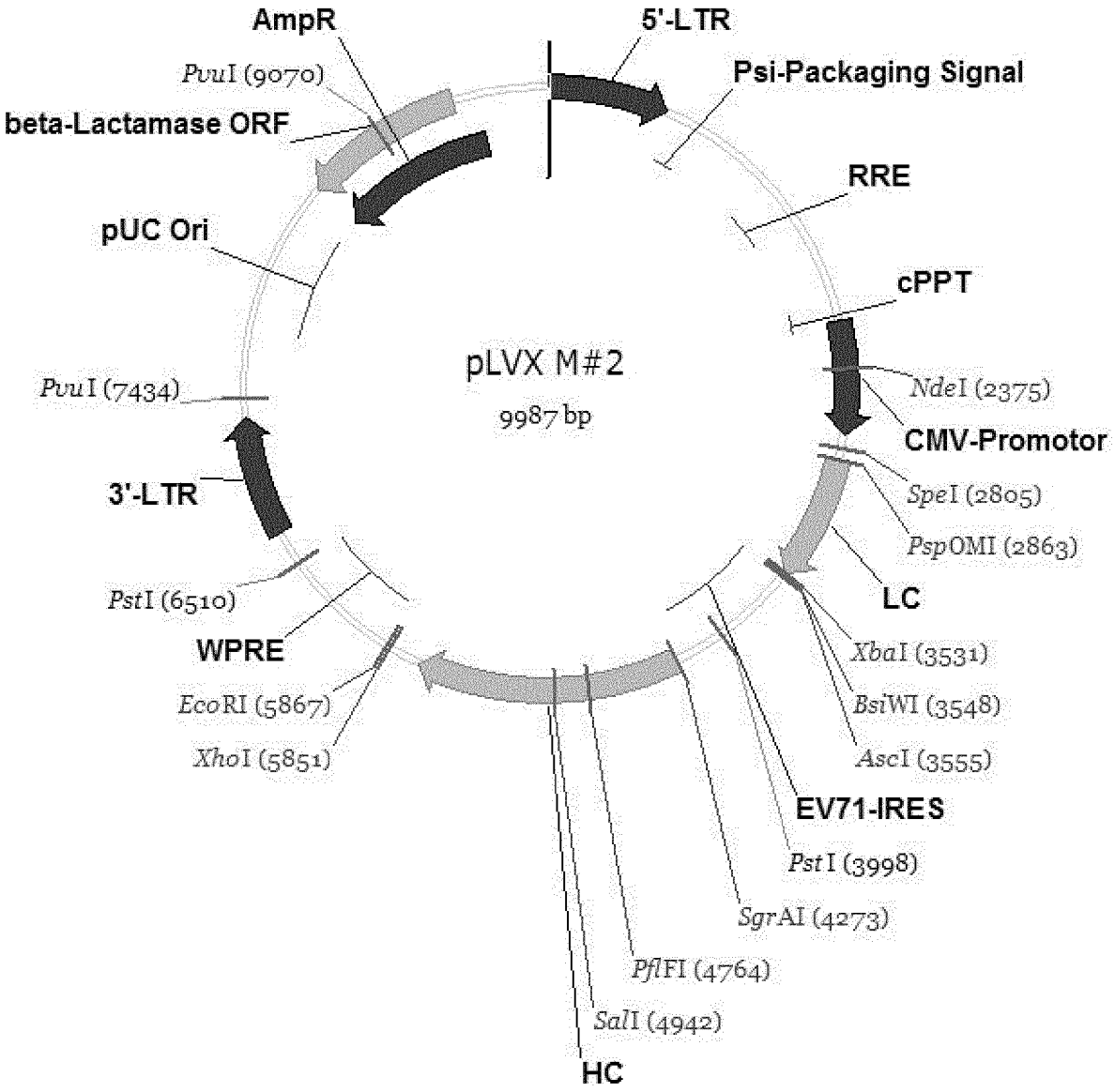

AmpR
5'-LTR
PvuI (9070)
Psi-Packaging Signal
beta-Lactamase ORF
RRE
pUC Ori
cPPT
pLVX M#2
9987 bp
PvuI (7434)
NdeI (2375)
CMV-Promotor
3'-LTR
SpeI (2805)
PspOMI (2863)
LC
PstI (6510)
WPRE
XbaI (3531)
EcoRI (5867)
BsiWI (3548)
XhoI (5851)
AscI (3555)
EV71-IRES
PstI (3998)
SgrAI (4273)
PflFI (4764)
SalI (4942)
HC

Figure 16

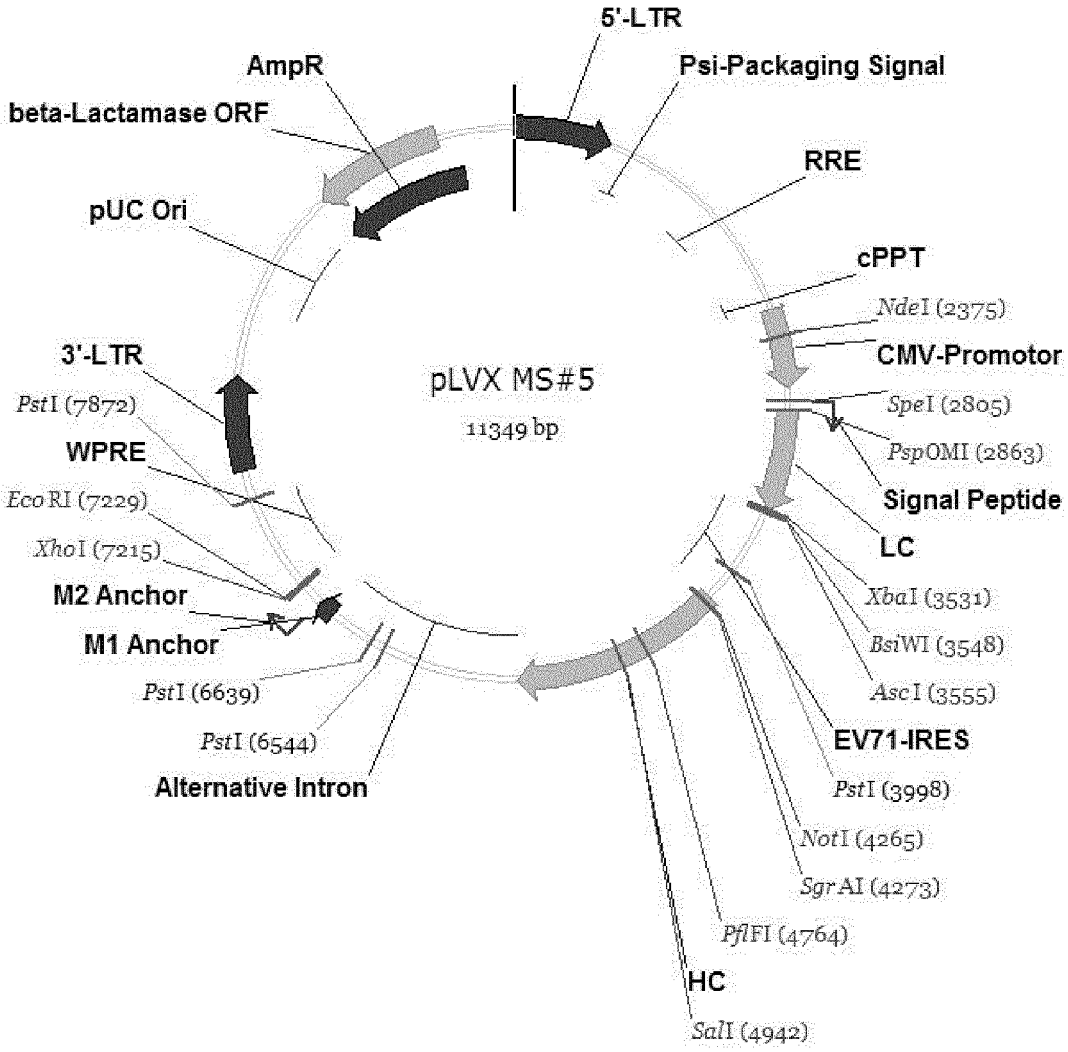

5'-LTR
Psi-Packaging Signal
RRE
cPPT
NdeI (2375)
CMV-Promotor
SpeI (2805)
PspOMI (2863)
Signal Peptide
LC
XbaI (3531)
BsiWI (3548)
AscI (3555)
EV71-IRES
PstI (3998)
NotI (4265)
SgrAI (4273)
PflFI (4764)
HC
SalI (4942)
Alternative Intron
PstI (6544)
PstI (6639)
M1 Anchor
M2 Anchor
XhoI (7215)
EcoRI (7229)
WPRE
PstI (7872)
3'-LTR
pUC Ori
beta-Lactamase ORF
AmpR
pLVX MS#5
11349 bp

Figure 17

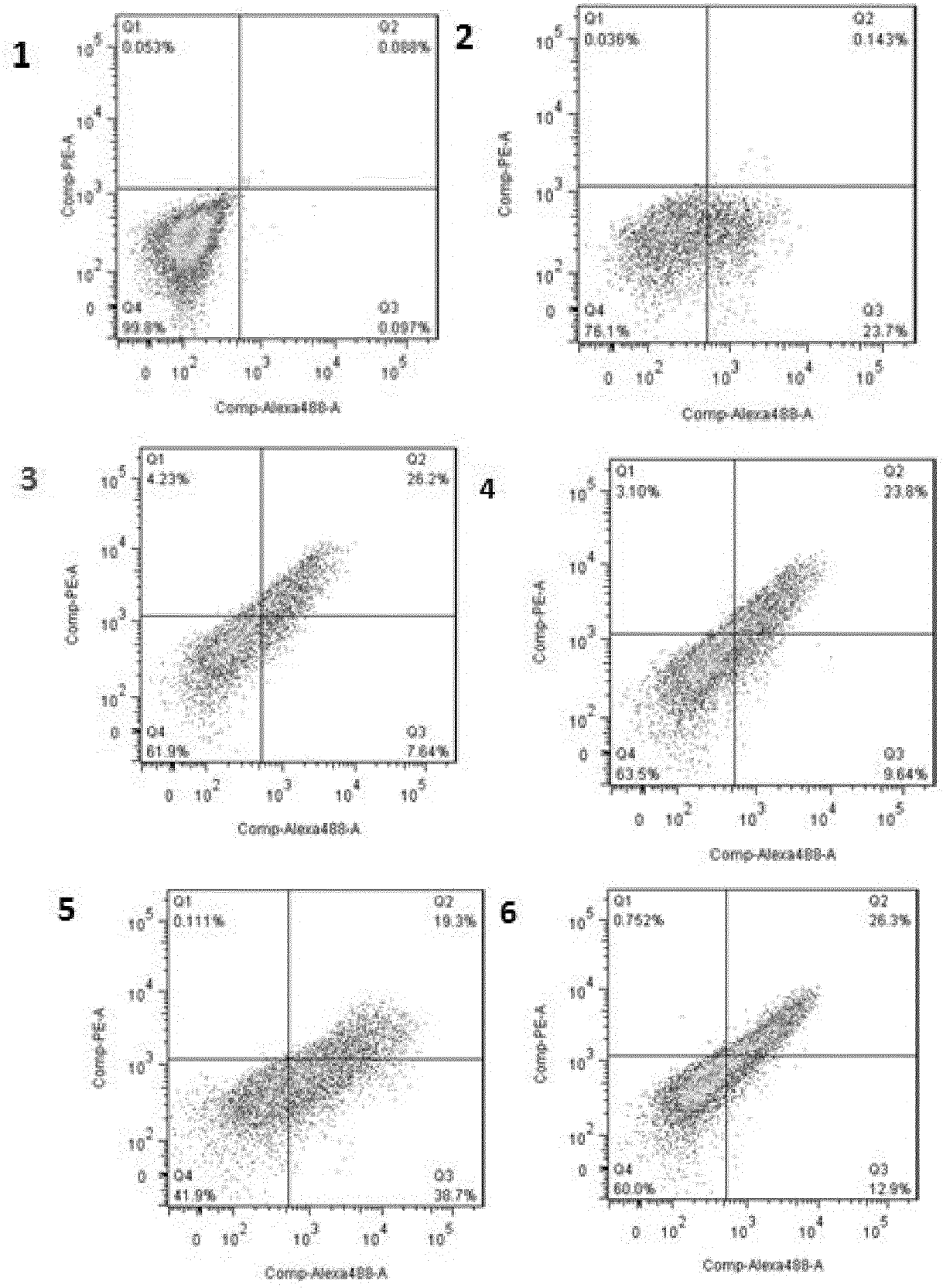

1
Q1
0.053%
Q2
0.088%
Q4
99.8%
Q3
0.097%
Comp-PE-A
Comp-Alexa488-A
2
Q1
0.036%
Q2
0.143%
Q4
76.1%
Q3
23.7%
Comp-PE-A
Comp-Alexa488-A
3
Q1
4.23%
Q2
26.2%
Q4
61.9%
Q3
7.64%
Comp-PE-A
Comp-Alexa488-A
4
Q1
3.10%
Q2
23.8%
Q4
63.5%
Q3
9.64%
Comp-PE-A
Comp-Alexa488-A
5
Q1
0.111%
Q2
19.3%
Q4
41.9%
Q3
38.7%
Comp-PE-A
Comp-Alexa488-A
6
Q1
0.752%
Q2
26.3%
Q4
60.0%
Q3
12.9%
Comp-PE-A
Comp-Alexa488-A

Figure 17 (continued)

