Figure 1

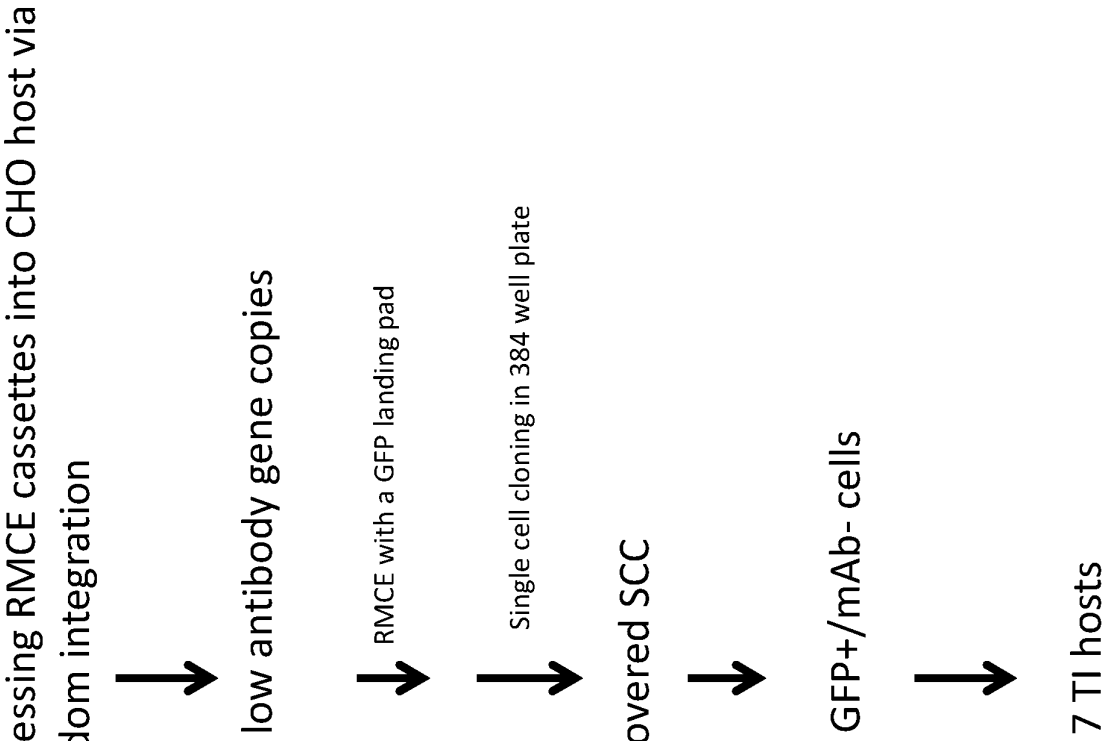
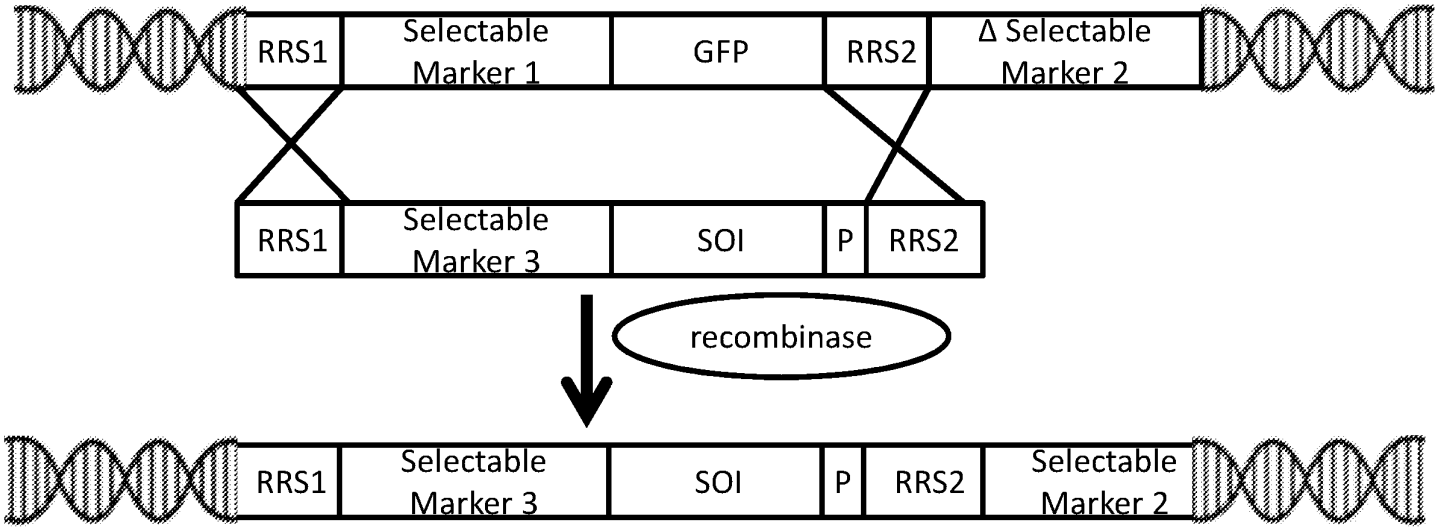
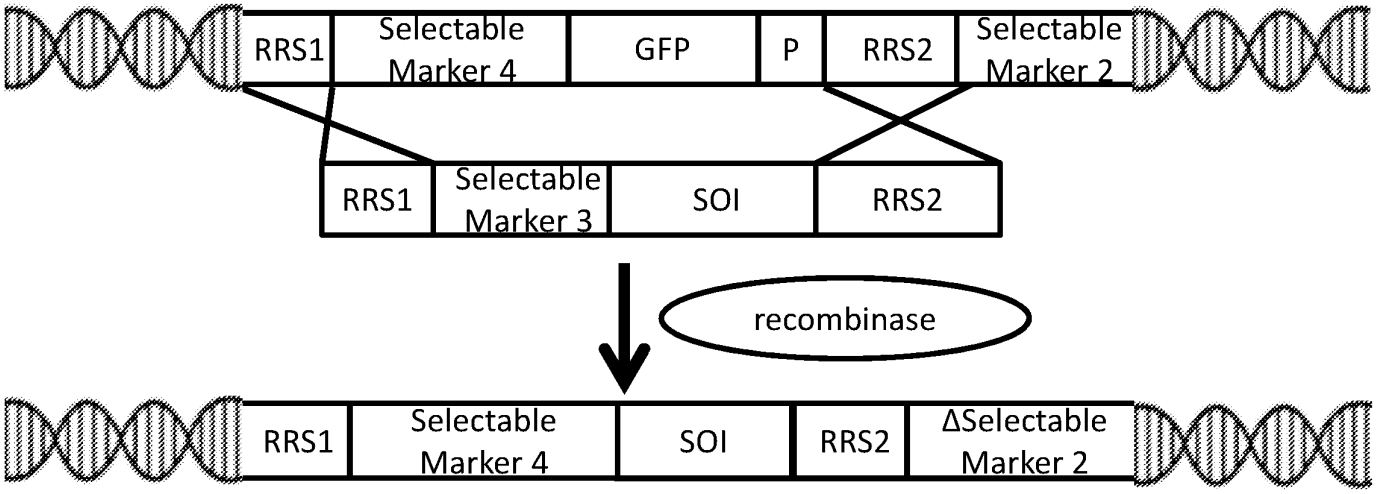

Introducing antibody expressing RMCE cassettes into CHO host via transposase-based or random integration
Identified clones with low antibody gene copies and high productivity
RMCE with a GFP landing pad
Single cell cloning in 384 well plate
Recovered SCC
Screened for GFP+/mAb- cells
7 TI hosts

Figure 2. Single-plasmid RMCE

A) Transposase generated TI hosts



B) Random integration generated TI hosts



P = promoter

Figure 3

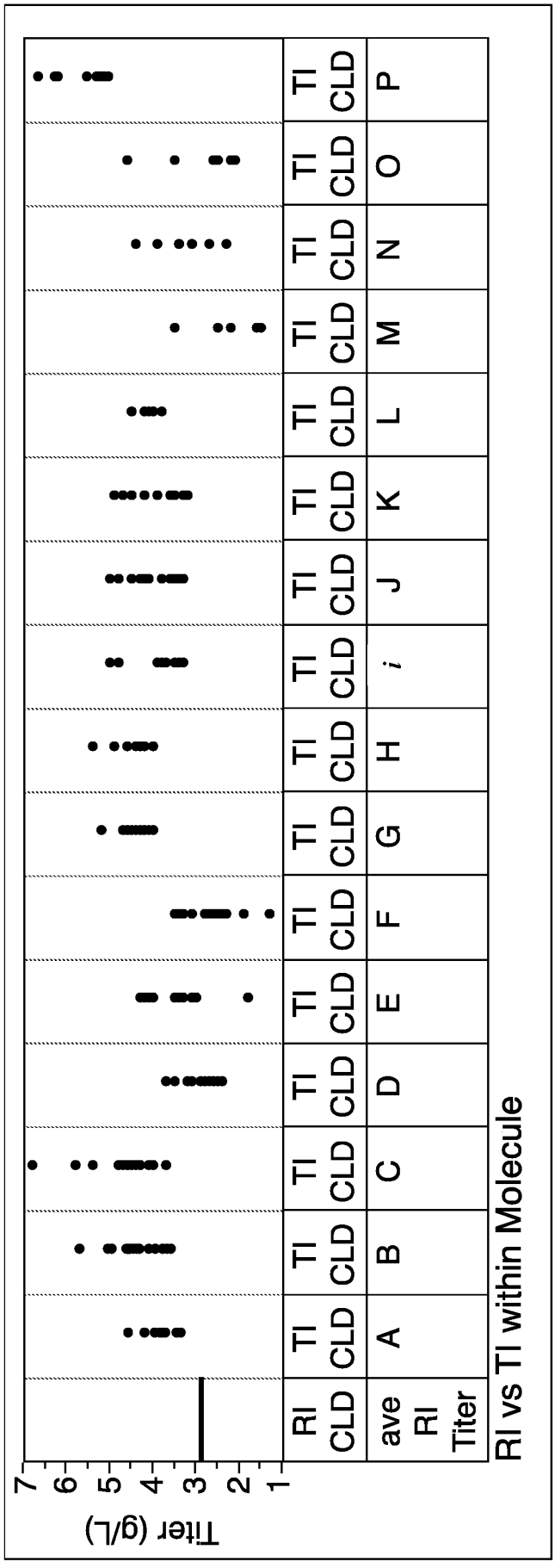
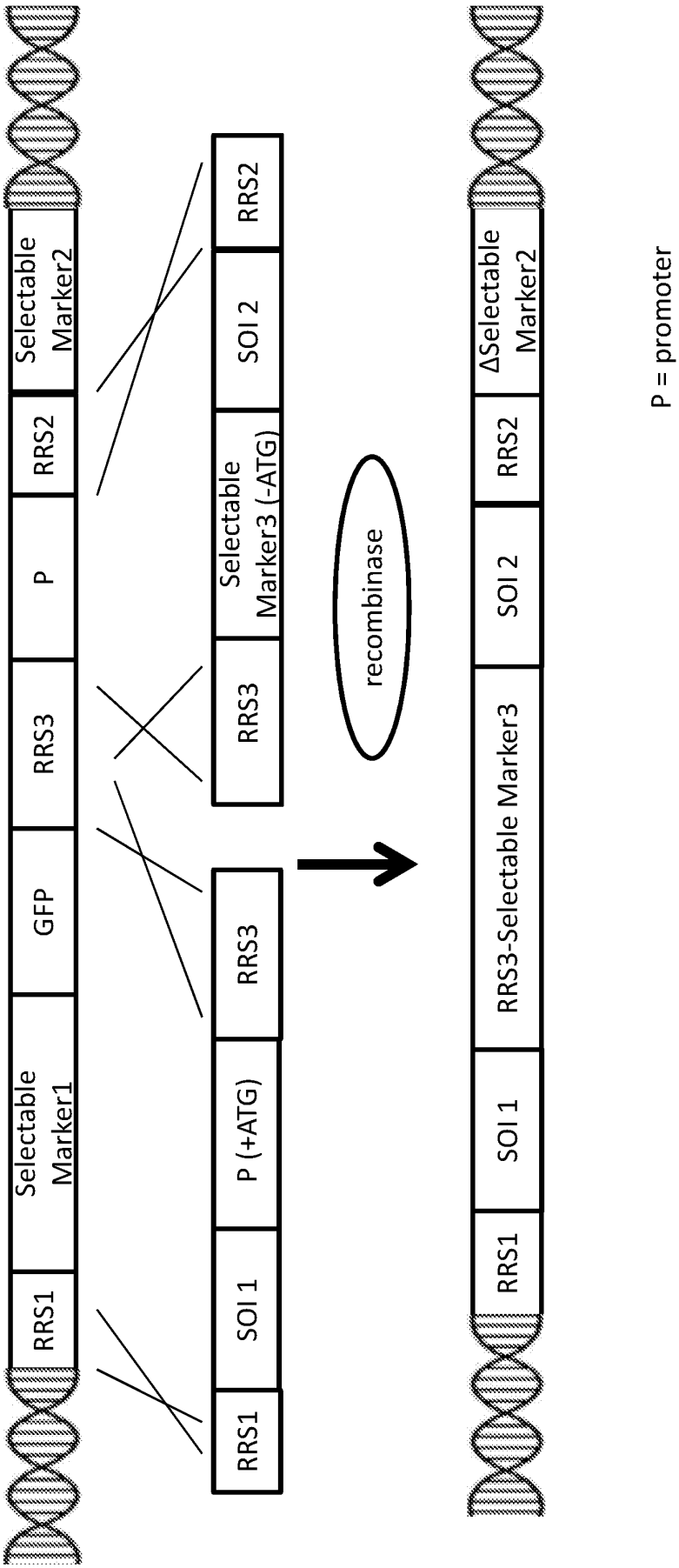

Day 14 titers in platform media and process condition without any optimization
Titer (g/L)
7
6
5
4
3
2
1
RI CLD
TI CLD
TI CLD
TI CLD
TI CLD
TI CLD
TI CLD
TI CLD
TI CLD
TI CLD
TI CLD
TI CLD
TI CLD
TI CLD
ave RI Titer
A
B
C
D
E
F
G
H
i
J
K
L
M
N
O
P
RI vs TI within Molecule

Figure 4. Two-plasmid RMCE



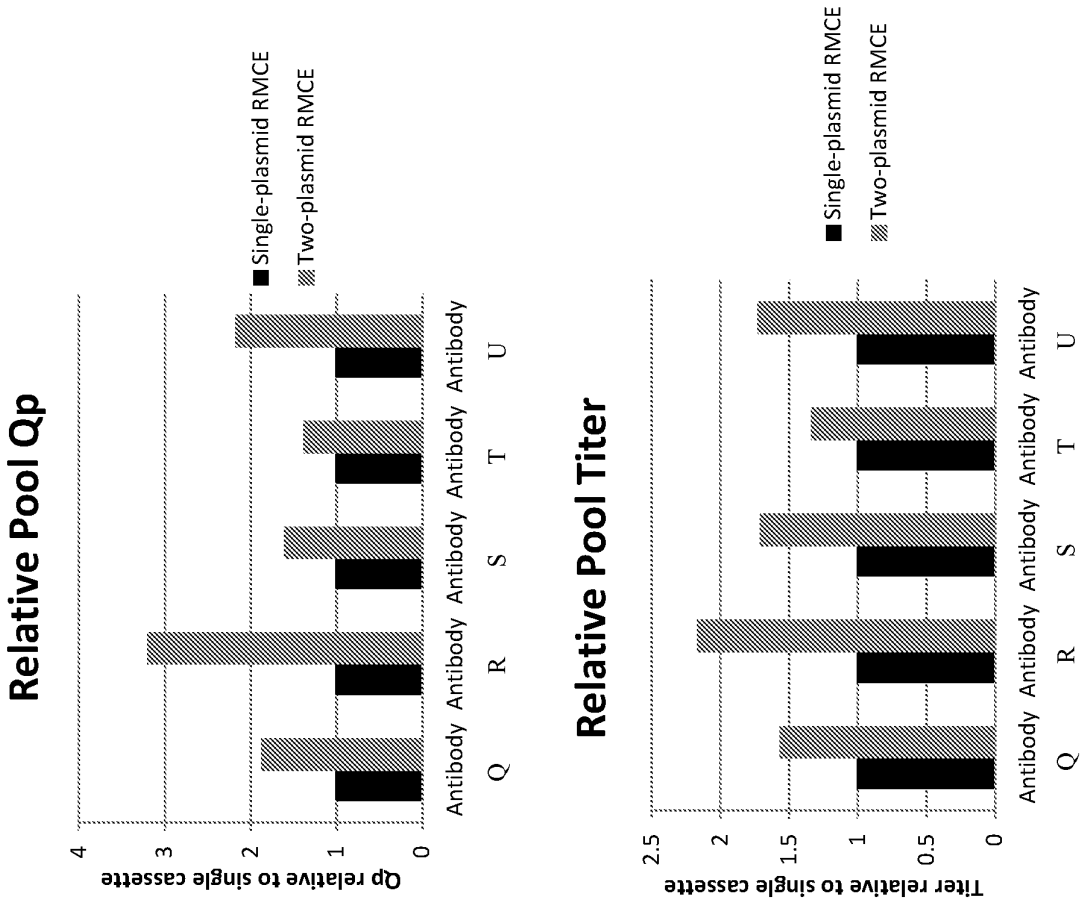
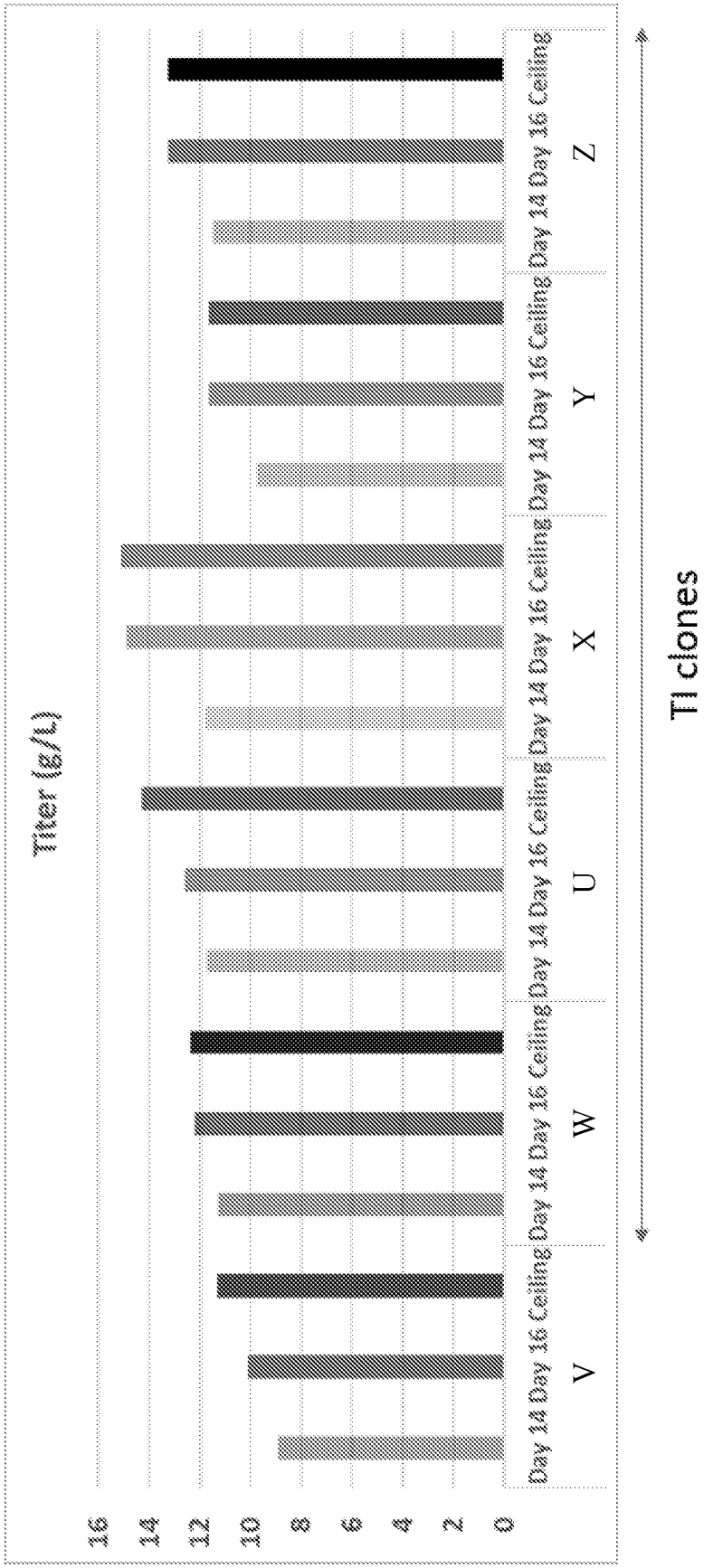
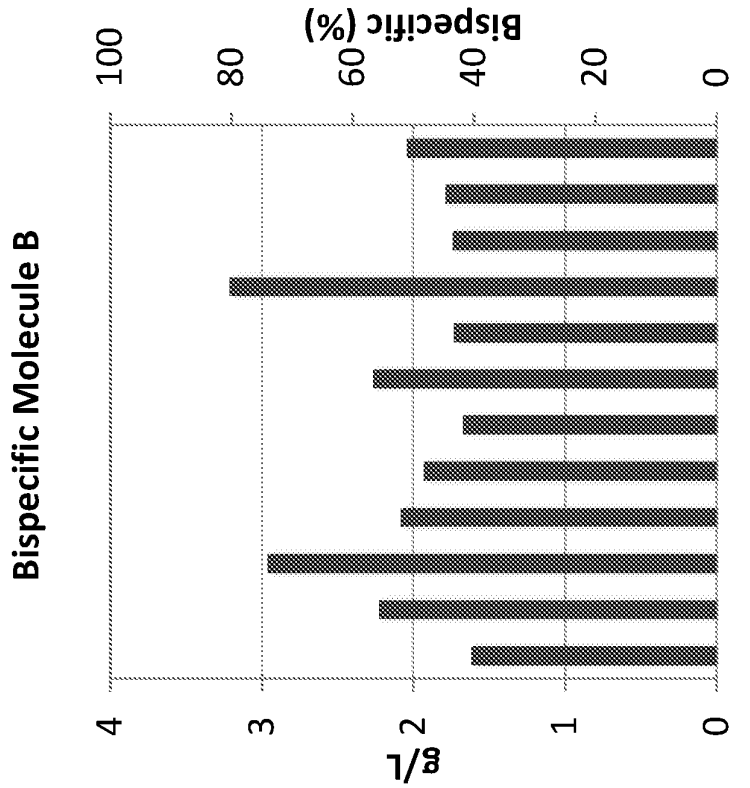

Relative Pool Qp
Qp relative to single cassette
0
1
2
3
4
Single-plasmid RMCE
Two-plasmid RMCE
Antibody Q
Antibody R
Antibody S
Antibody T
Antibody U
Relative Pool Titer
Titer relative to single cassette
0
0.5
1
1.5
2
2.5
Single-plasmid RMCE
Two-plasmid RMCE
Antibody Q
Antibody R
Antibody S
Antibody T
Antibody U

Figure 5.

Figure 6.


Titer (g/L)
16
14
12
10
8
6
4
2
0
Day 14
Day 16 Ceiling
V
W
U
X
Y
Z
TI clones


Bispecific Molecule B
Bispecific (%)
g/L
0
20
40
60
80
100
0
1
2
3
4

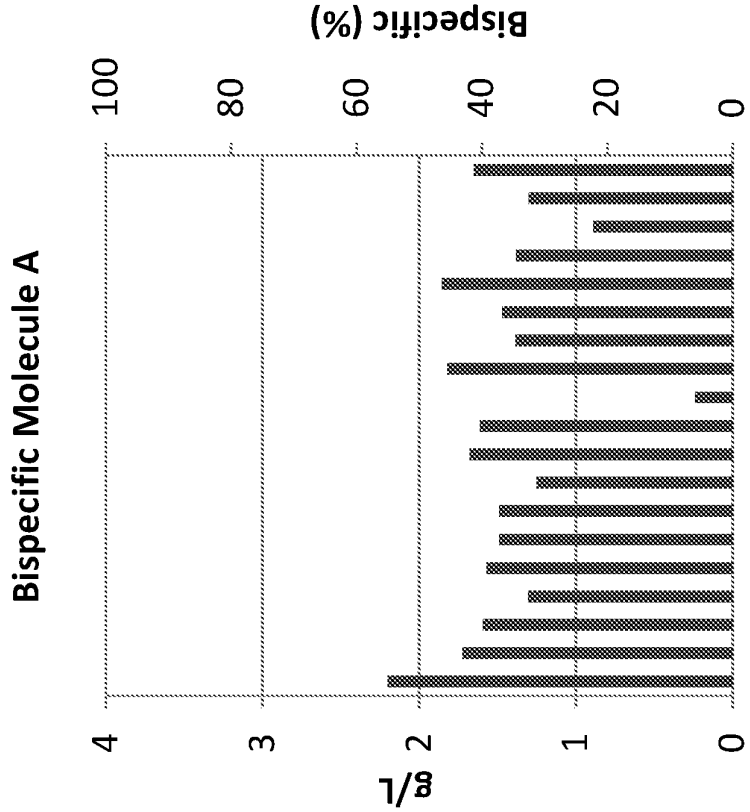

Bispecific Molecule A
Bispecific (%)
g/L
0
20
40
60
80
100
0
1
2
3
4

Figure 7.

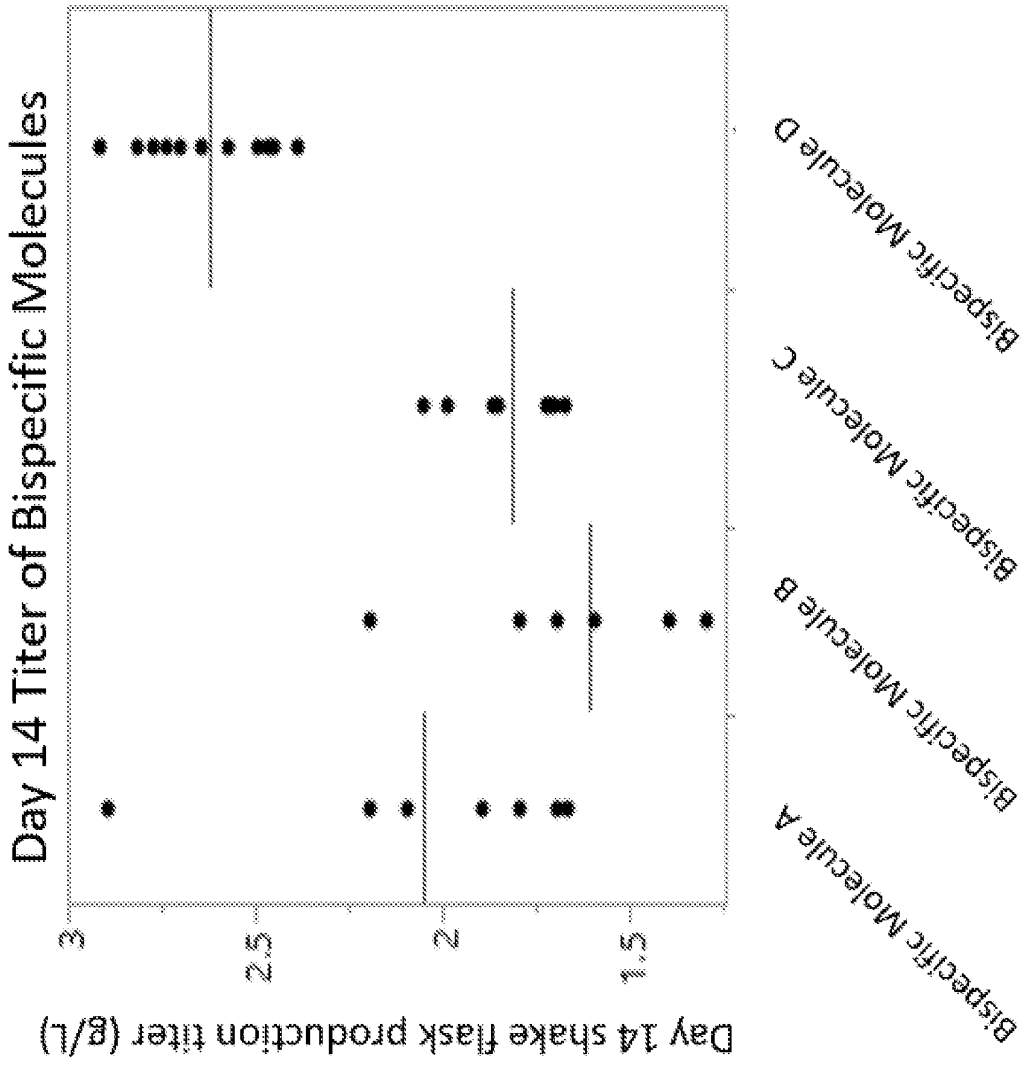

Day 14 Titer of Bispecific Molecules
Day 14 shake flask production titer (g/L)
3
2.5
2
1.5
Bispecific Molecule A
Bispecific Molecule B
Bispecific Molecule C
Bispecific Molecule D

Figure 8.

Figure 9A.

Molecule	# of Cell line developments	cell lines screened	Titers observed
mAb-I (Difficult to express molecule)	4 attempts	120+	Below 50% of typical range
mAB-II (Average expressing molecule)	1 attempt	24	Typical range (>2g/L)

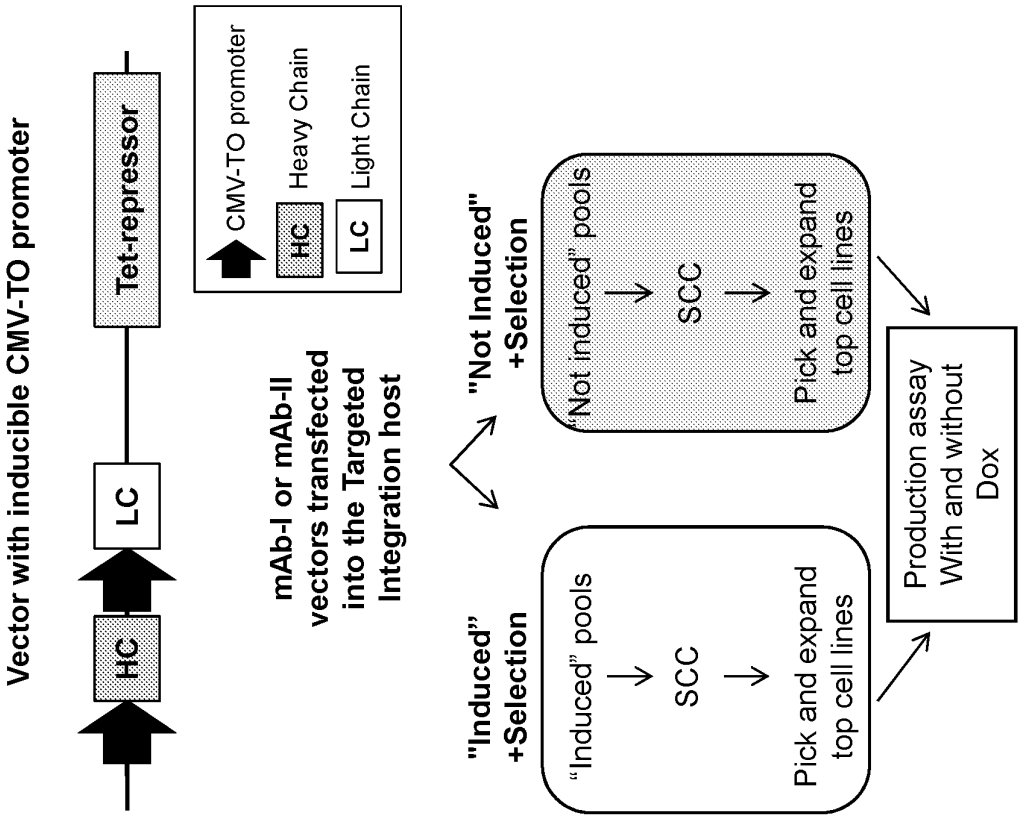

Vector with inducible CMV-TO promoter
HC
LC
Tet-repressor
CMV-TO promoter
HC
Heavy Chain
LC
Light Chain
mAb-I or mAb-II vectors transfected into the Targeted Integration host
"Induced" +Selection
"Not Induced" +Selection
"Induced" pools
SCC
Pick and expand top cell lines
"Not induced" pools
SCC
Pick and expand top cell lines
Production assay With and without Dox

Figure 9B.

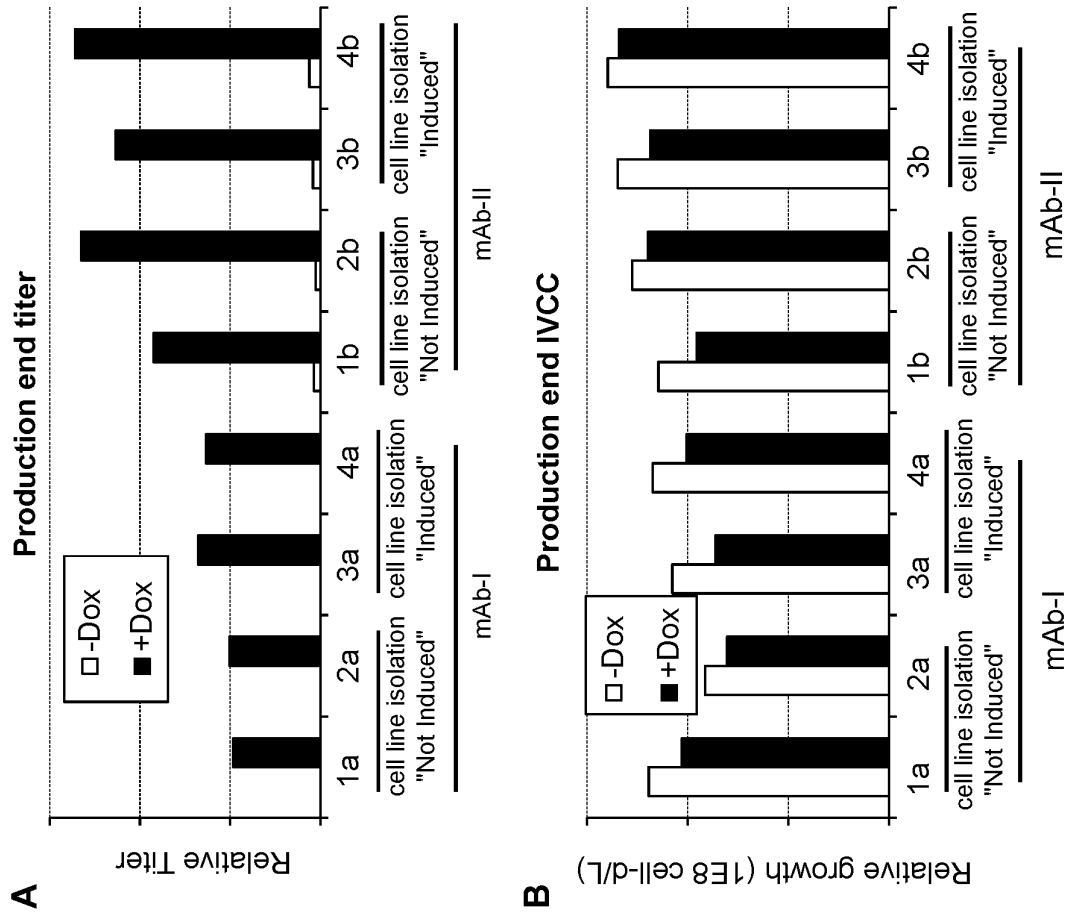

A
Production end titer
Relative Titer
□-Dox
■+Dox
1a
2a
3a
4a
1b
2b
3b
4b
cell line isolation "Not Induced"
cell line isolation "Induced"
cell line isolation "Not Induced"
cell line isolation "Induced"
mAb-I
mAb-II
B
Production end IVCC
Relative growth (1E8 cell-d/L)
□-Dox
■+Dox
1a
2a
3a
4a
1b
2b
3b
4b
cell line isolation "Not Induced"
cell line isolation "Induced"
cell line isolation "Not Induced"
cell line isolation "Induced"
mAb-I
mAb-II

Figure 10.

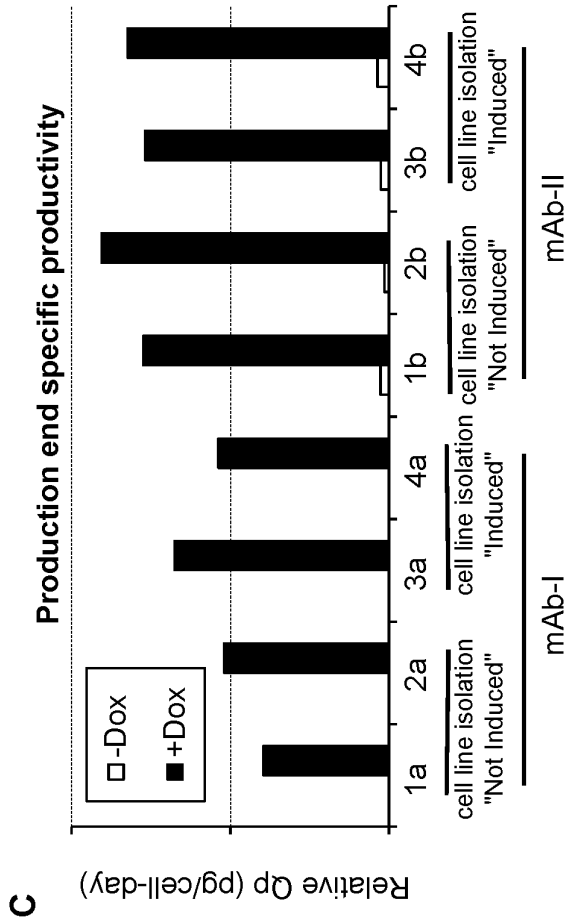


Figure 10 (continue).

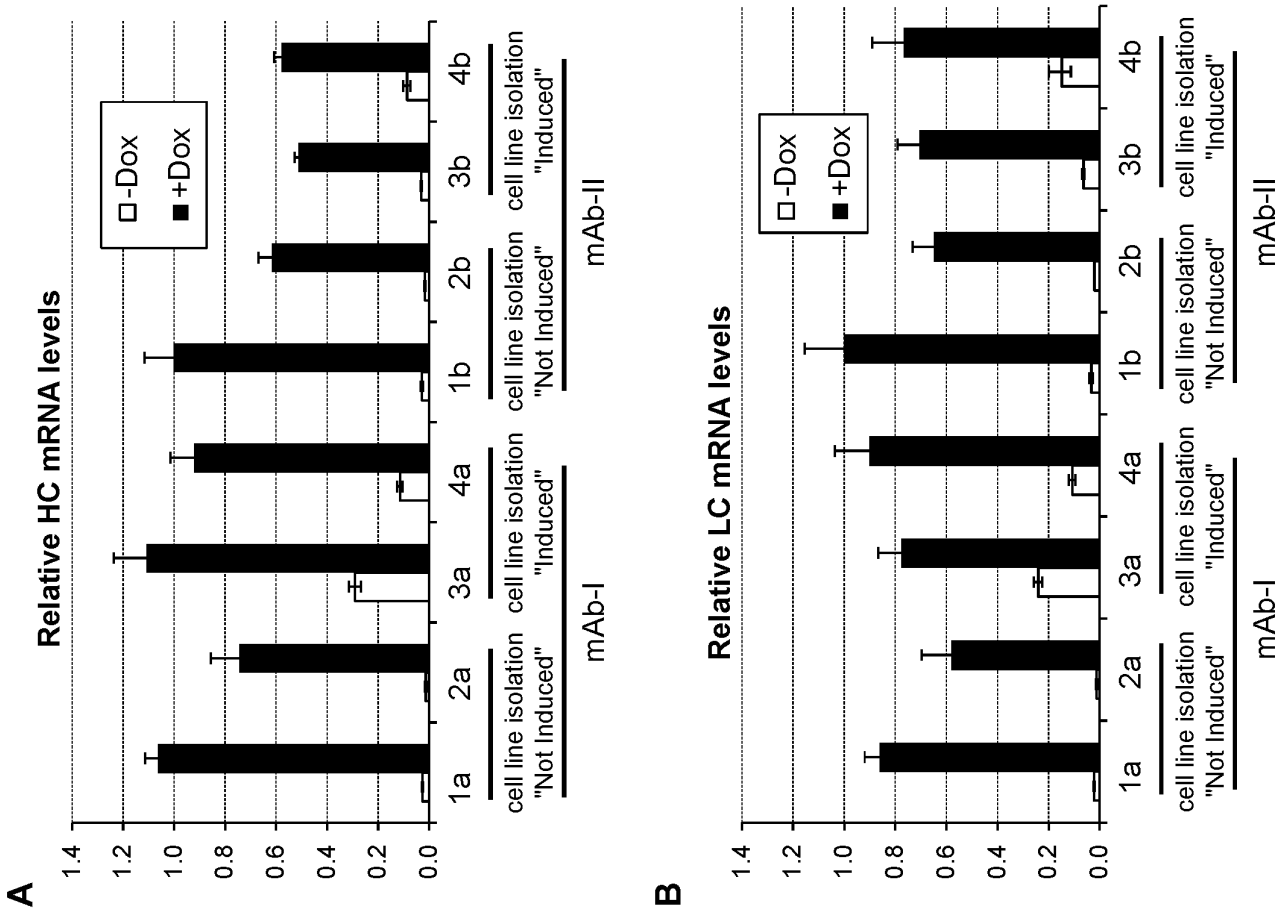

A
Relative HC mRNA levels
□-Dox
■+Dox
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1a
2a
3a
4a
1b
2b
3b
4b
cell line isolation "Not Induced"
cell line isolation "Induced"
cell line isolation "Not Induced"
cell line isolation "Induced"
mAb-I
mAb-II
B
Relative LC mRNA levels
□-Dox
■+Dox
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1a
2a
3a
4a
1b
2b
3b
4b
cell line isolation "Not Induced"
cell line isolation "Induced"
cell line isolation "Not Induced"
cell line isolation "Induced"
mAb-I
mAb-II

Figure 11.

Figure 12.

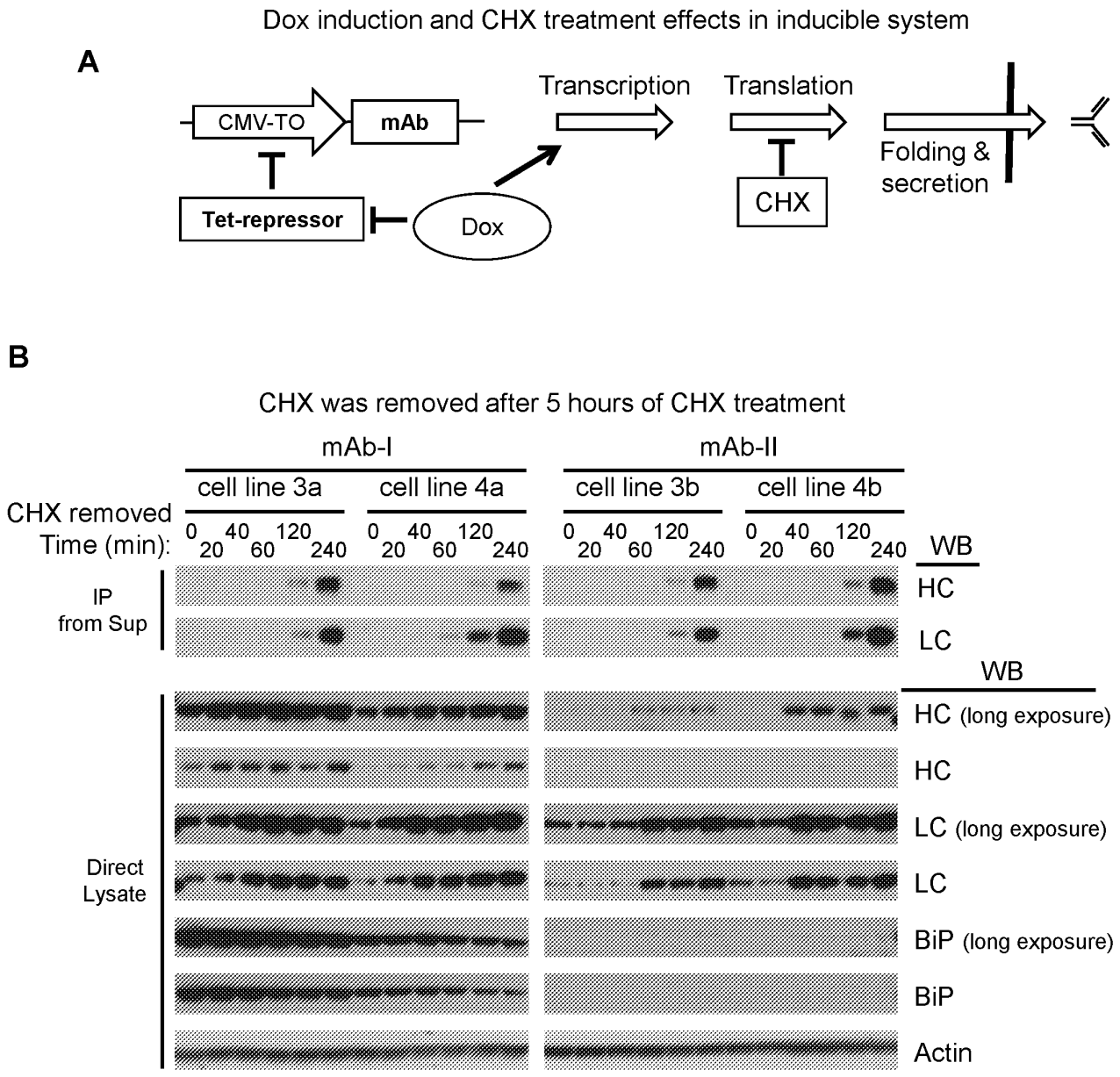

Dox induction and CHX treatment effects in inducible system
A
CMV-TO
mAb
Tet-repressor
Dox
Transcription
Translation
CHX
Folding & secretion
B
CHX was removed after 5 hours of CHX treatment
mAb-I
mAb-II
cell line 3a
cell line 4a
cell line 3b
cell line 4b
CHX removed
Time (min):
0 20 40 60 120 240
WB
IP from Sup
HC
LC
Direct Lysate
HC (long exposure)
HC
LC (long exposure)
LC
BiP (long exposure)
BiP
Actin

Figure 12 (continue).

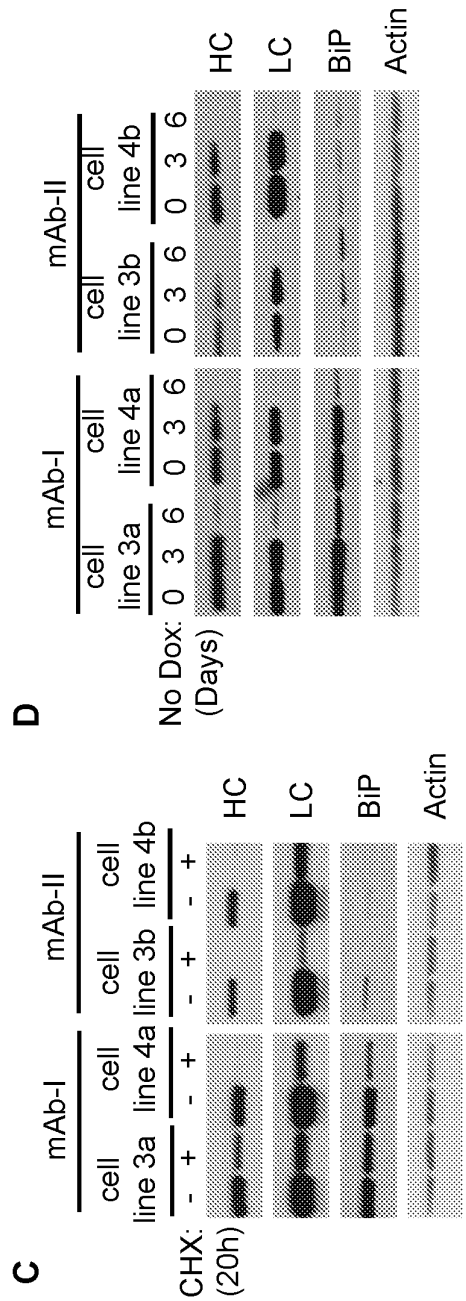


Figure 13.

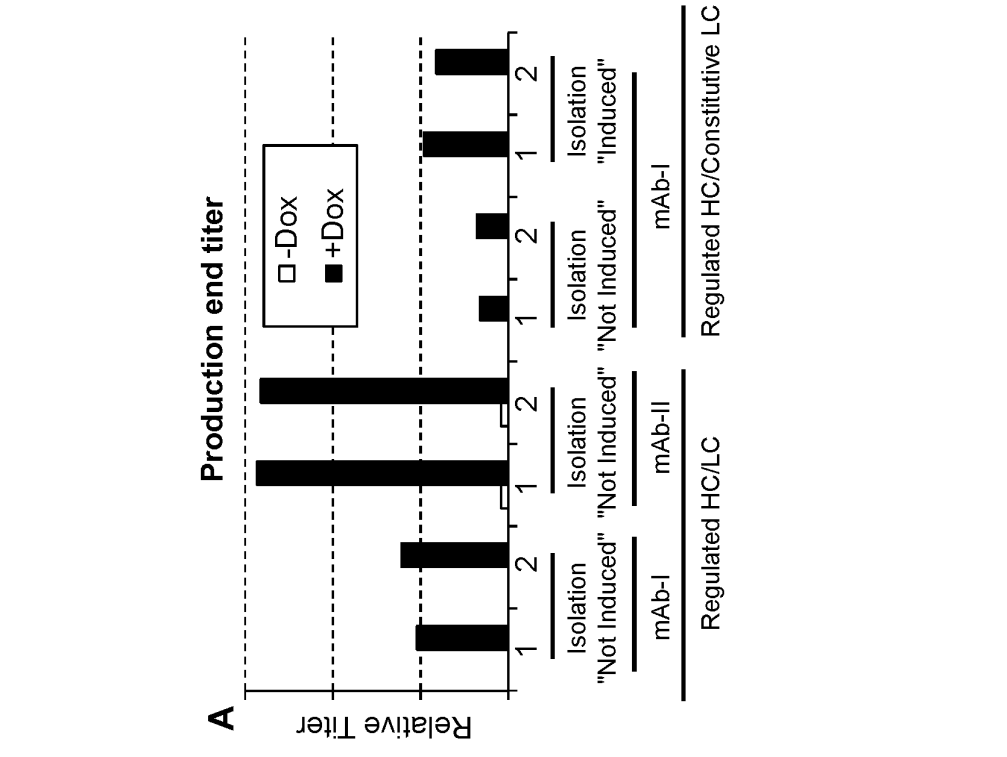
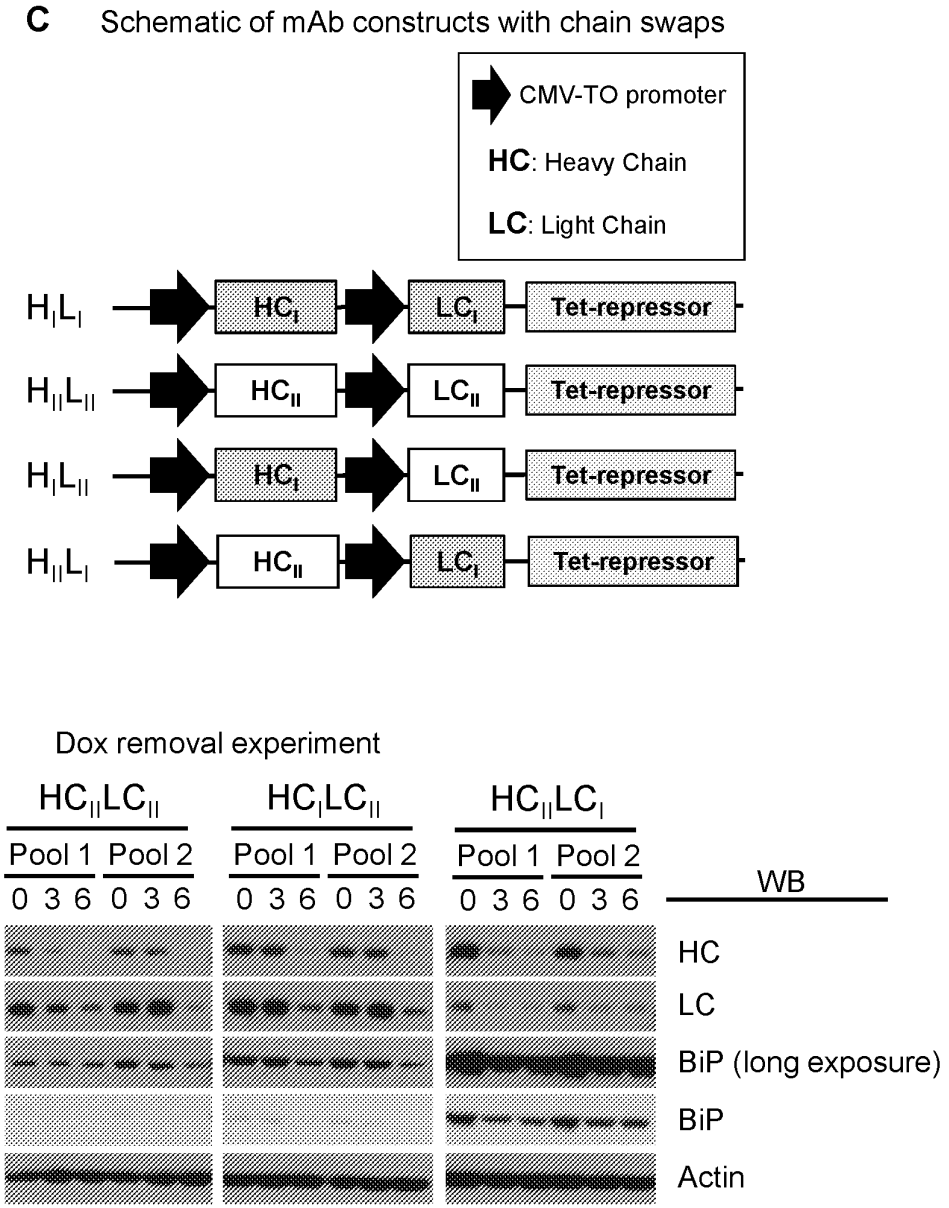

A
Production end titer
Relative Titer
-Dox
+Dox
1
2
Isolation "Not Induced"
mAb-I
Isolation "Not Induced"
mAb-II
Regulated HC/LC
Isolation "Not Induced"
mAb-I
Isolation "Induced"
Regulated HC/Constitutive LC
B
Isolation "Not Induced"
Regulated HC/LC
Regulated HC Constitutive LC
mAb-I
mAb-II
mAb-I
Pool:
1 2 1 2 1 2
-Dox
+Dox
HC
LC
BiP
Actin

Figure 13 (continue).



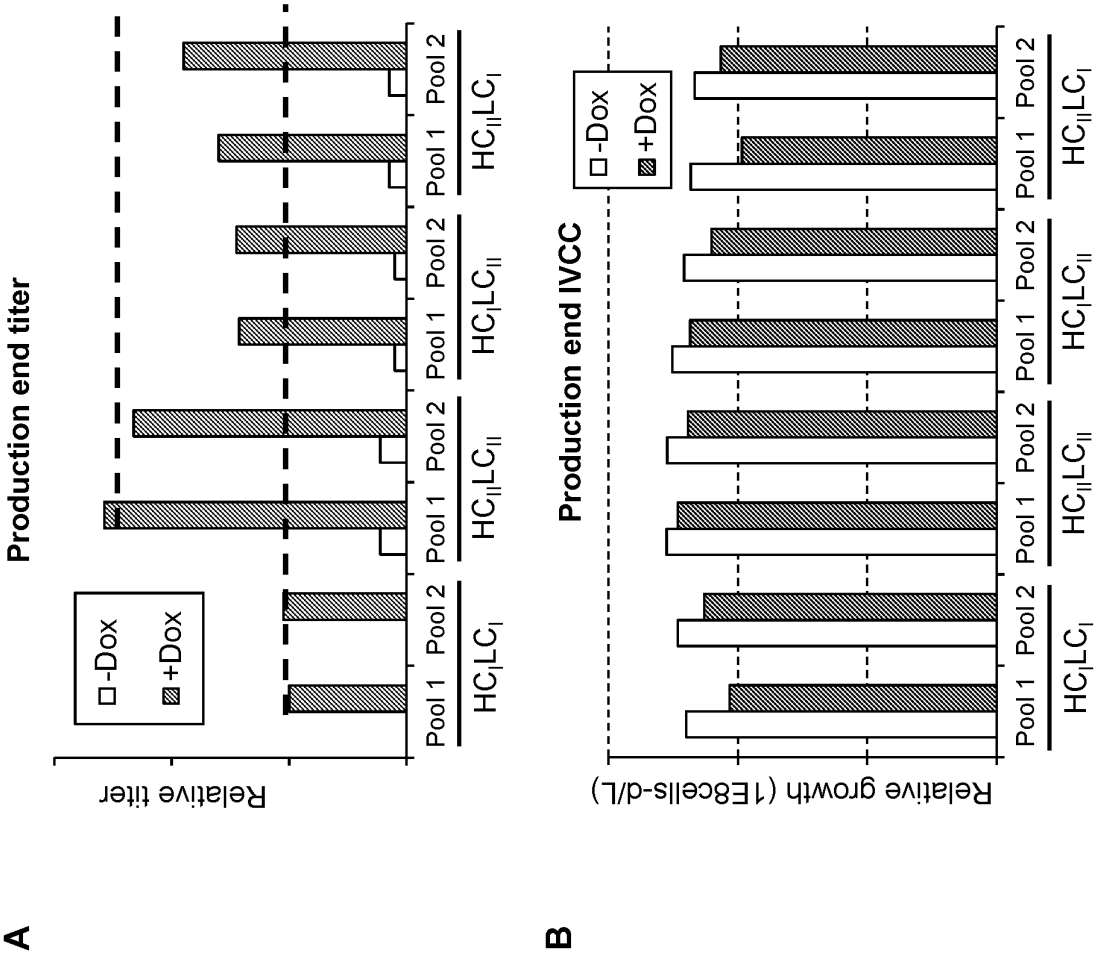
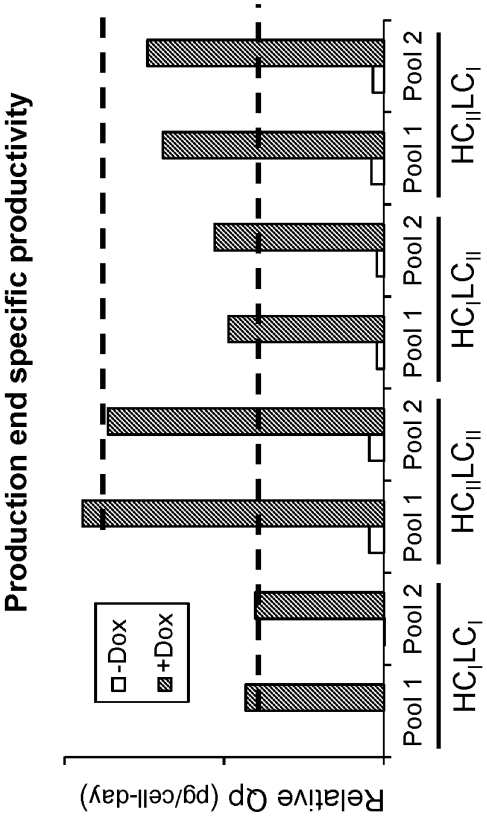

A
Production end titer
Relative titer
-Dox
+Dox
Pool 1
Pool 2
HCILCI
HCIILCII
HCILCII
HCIILCI
B
Production end IVCC
Relative growth (1E8cells-d/L)

Figure 14.

Figure 14 (continue).

C



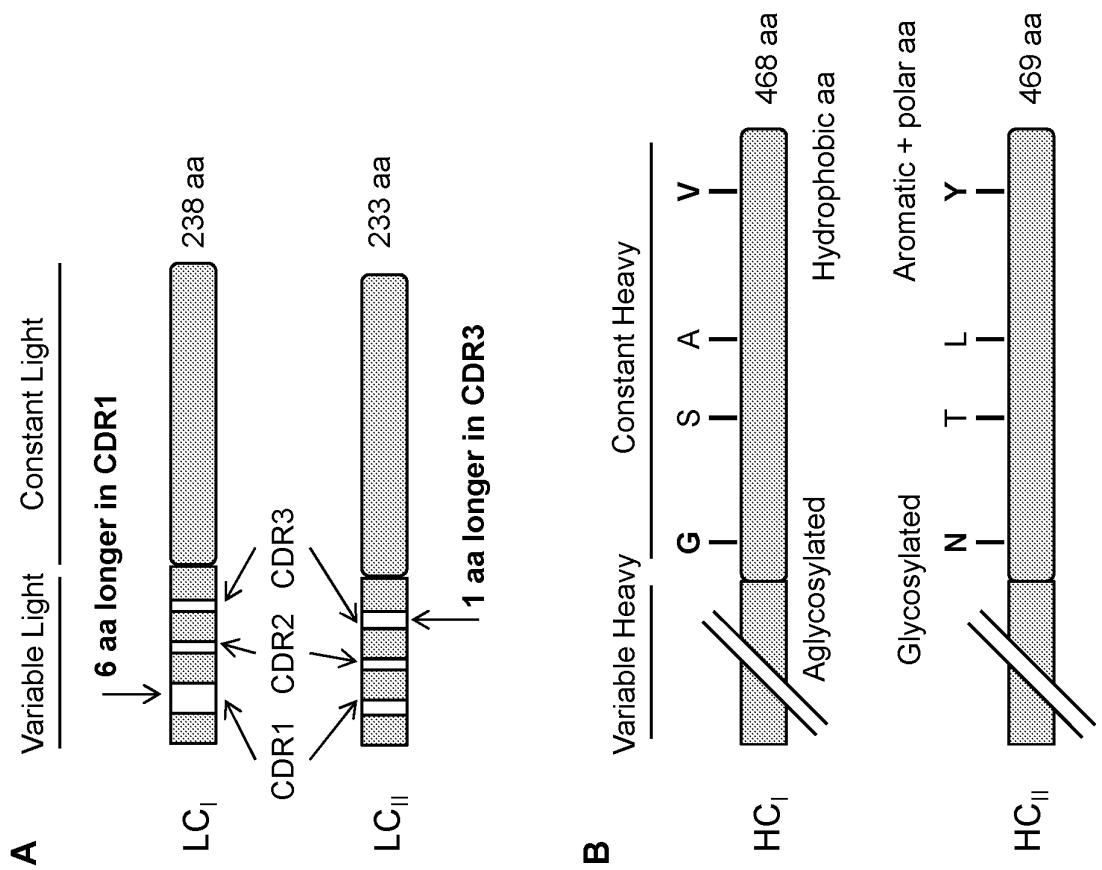

A
Variable Light
Constant Light
6 aa longer in CDR1
238 aa
233 aa
CDR1
CDR2
CDR3
LC$_I$
LC$_{II}$
1 aa longer in CDR3
B
Variable Heavy
Constant Heavy
G
S
A
V
468 aa
Aglycosylated
Hydrophobic aa
HC$_I$
Glycosylated
N
T
L
Y
Aromatic + polar aa
469 aa
HC$_{II}$

Figure 15.

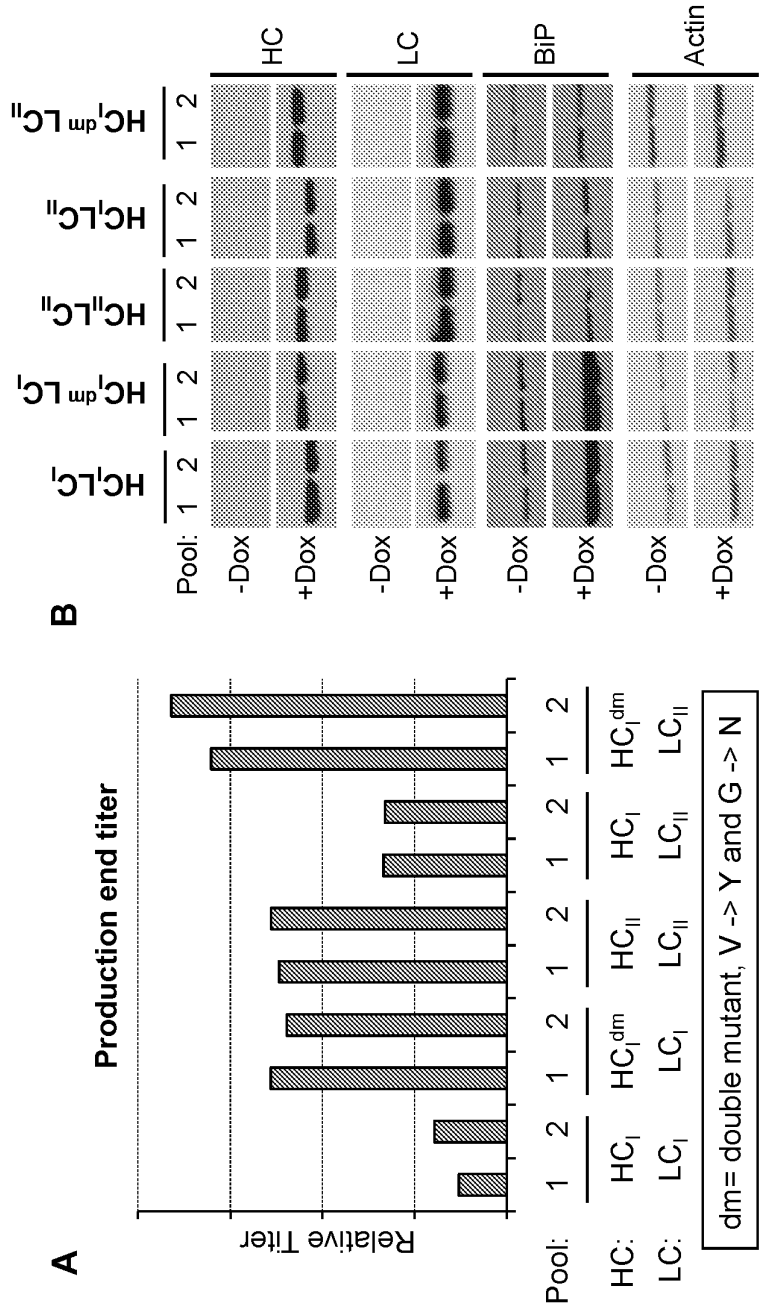

A
Production end titer
Relative Titer
Pool: 1 2 1 2 1 2 1 2 1 2 1 2
HC: HC$_I$ HC$_I^{dm}$ HC$_{II}$ HC$_I$ HC$_I^{dm}$
LC: LC$_I$ LC$_I$ LC$_{II}$ LC$_{II}$
dm= double mutant, V -> Y and G -> N
B
HC$_I$LC$_I$
HC$_I^{dm}$LC$_I$
HC$_{II}$LC$_{II}$
HC$_I$LC$_{II}$
HC$_I^{dm}$LC$_{II}$
Pool: 1 2 1 2 1 2 1 2 1 2
-Dox
+Dox
HC
LC
BiP
Actin

Figure 16.

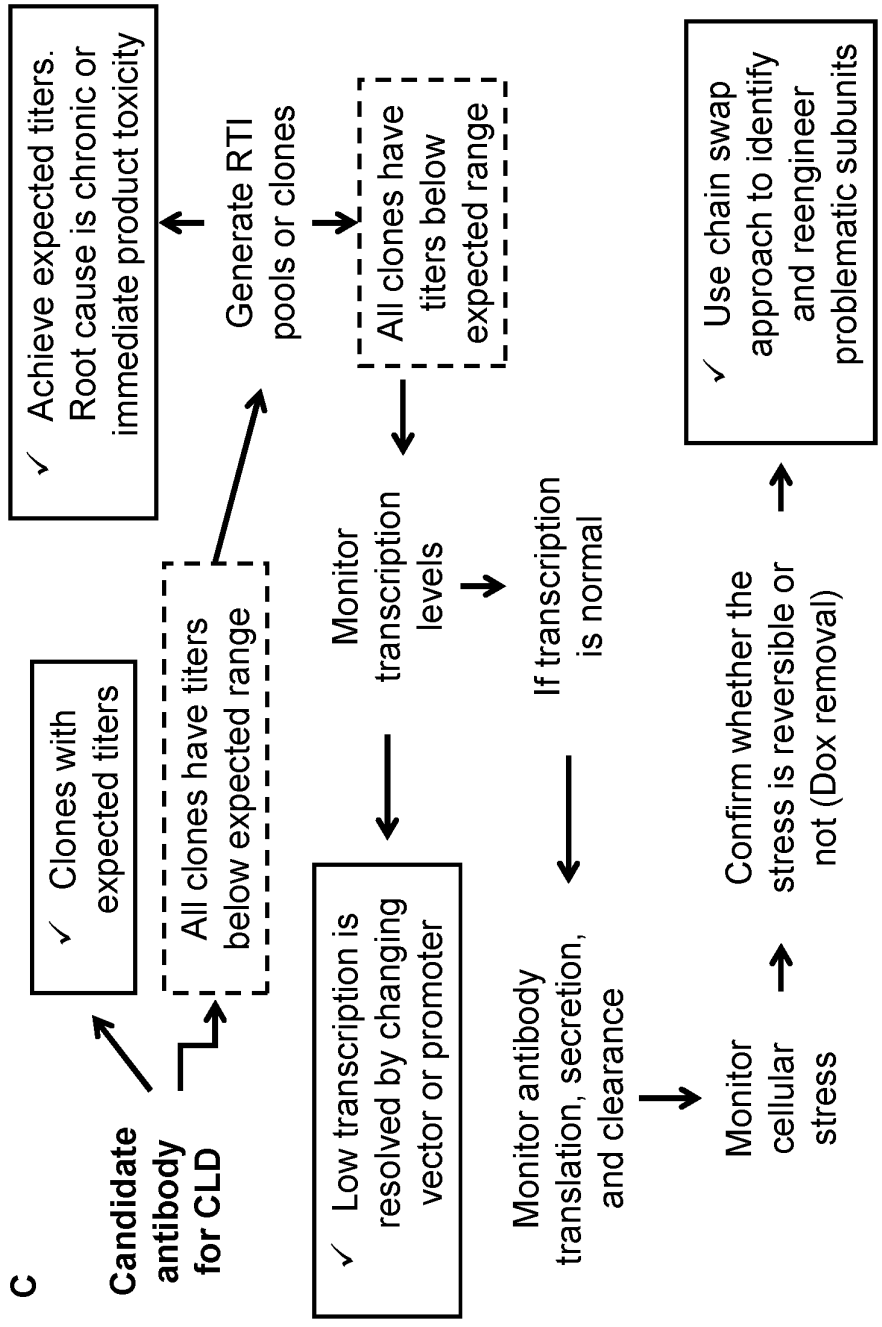
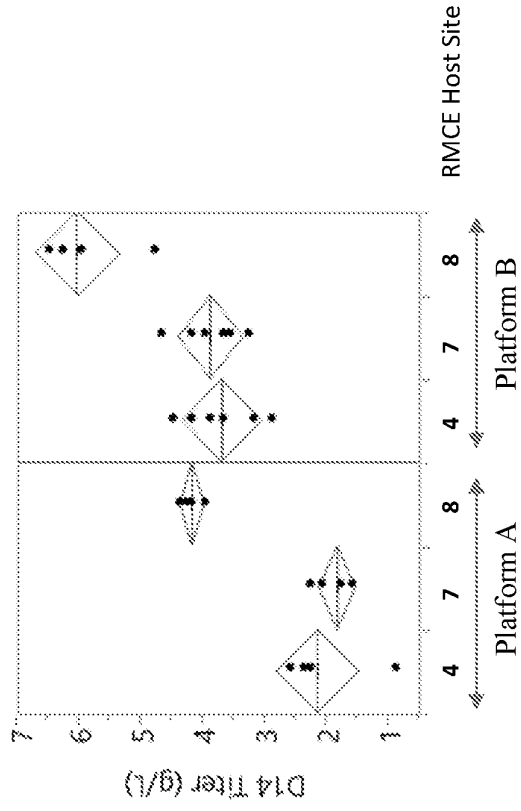


Figure 16 (continue).


D14 Titer (g/L)
7
6
5
4
3
2
1
4
7
8
Platform A
4
7
8
Platform B
RMCE Host Site

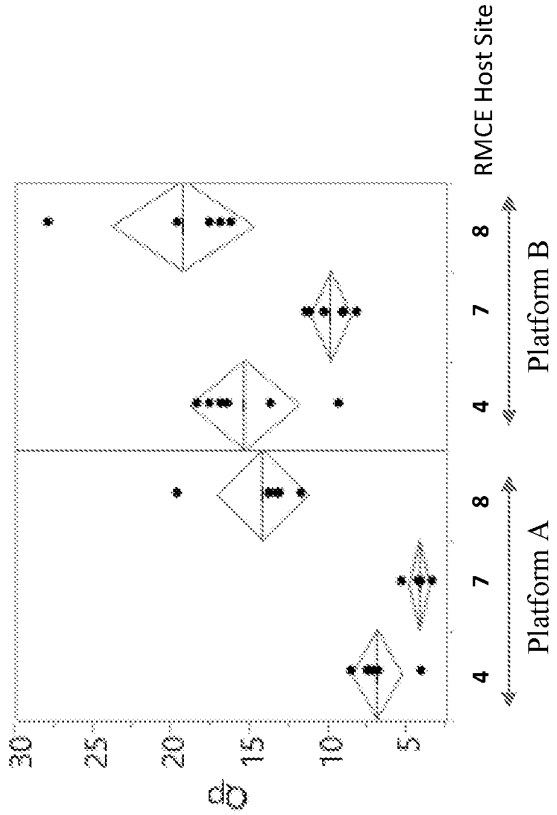

Qp
30
25
20
15
10
5
4
7
8
Platform A
4
7
8
Platform B
RMCE Host Site

Figure 17.

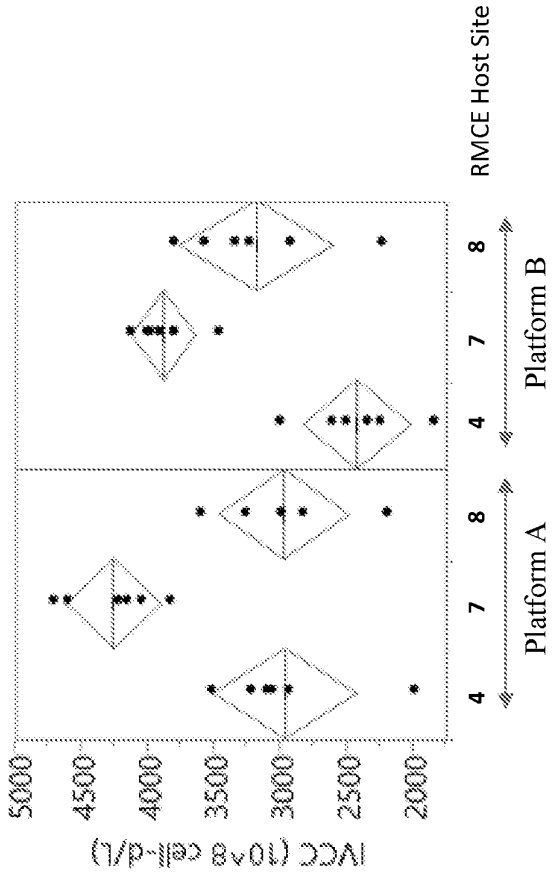


Figure 17 (continue).

Figure 18.

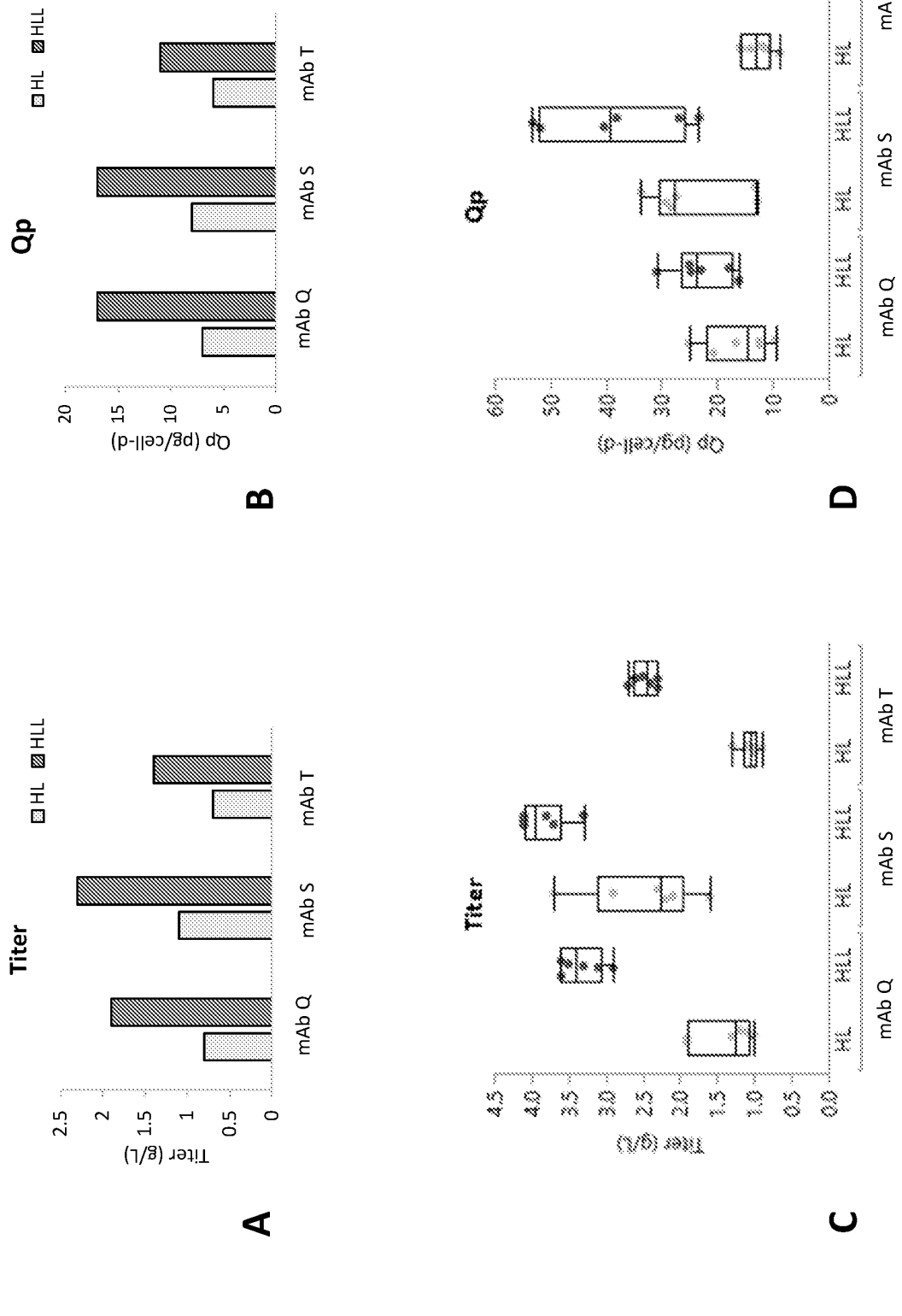

Titer
HL
HLL
Titer (g/L)
2.5
2
1.5
1
0.5
0
mAb Q
mAb S
mAb T
A
Qp
HL
HLL
Qp (pg/cell-d)
20
15
10
5
0
mAb Q
mAb S
mAb T
B
Titer
Titer (g/L)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
HL
HLL
HL
HLL
HL
HLL
mAb Q
mAb S
mAb T
C
Qp
Qp (pg/cell-d)
60
50
40
30
20
10
0
HL
HLL
HL
HLL
HL
HLL
mAb Q
mAb S
mAb T
D

Figure 19.

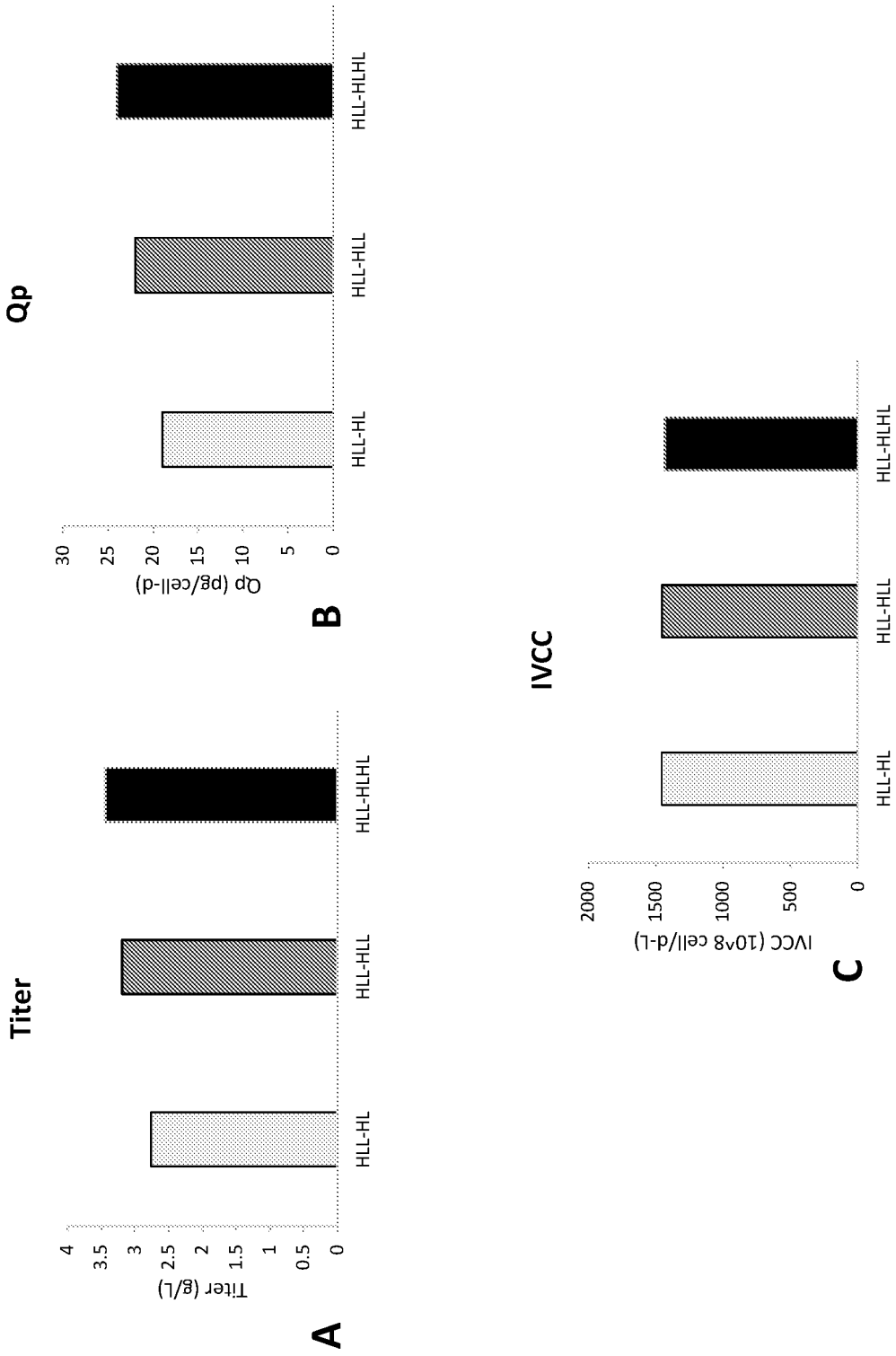
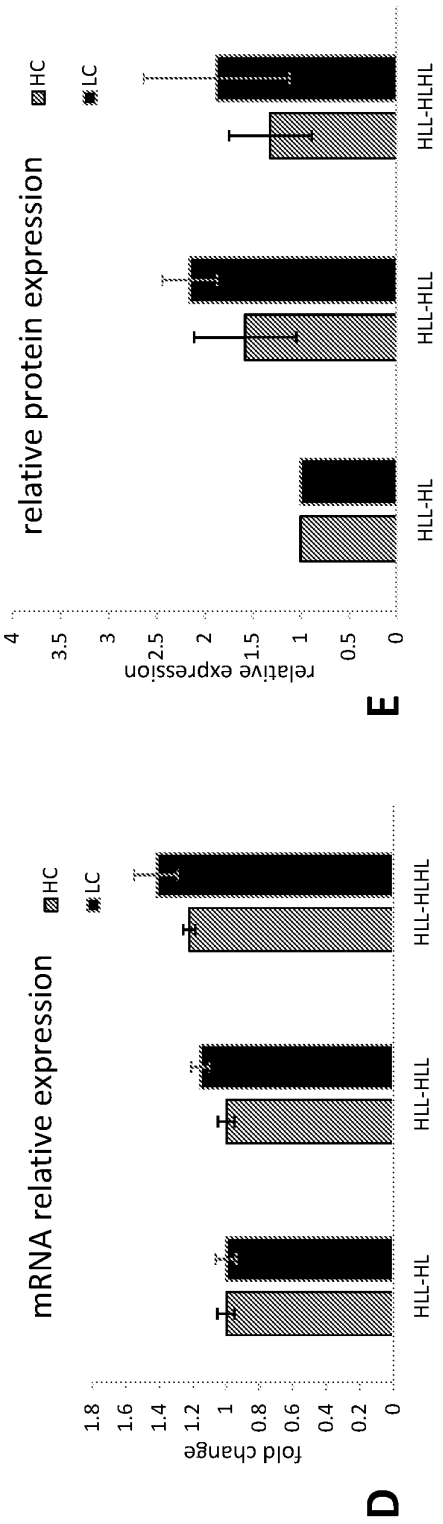

Titer
Titer (g/L)
0
0.5
1
1.5
2
2.5
3
3.5
4
HLL-HL
HLL-HLL
HLL-HLHL
A
Qp
Qp (pg/cell-d)
0
5
10
15
20
25
30
HLL-HL
HLL-HLL
HLL-HLHL
B
IVCC
IVCC (10^8 cell/d-L)
0
500
1000
1500
2000
HLL-HL
HLL-HLL
HLL-HLHL
C

Figure 19 (continued).



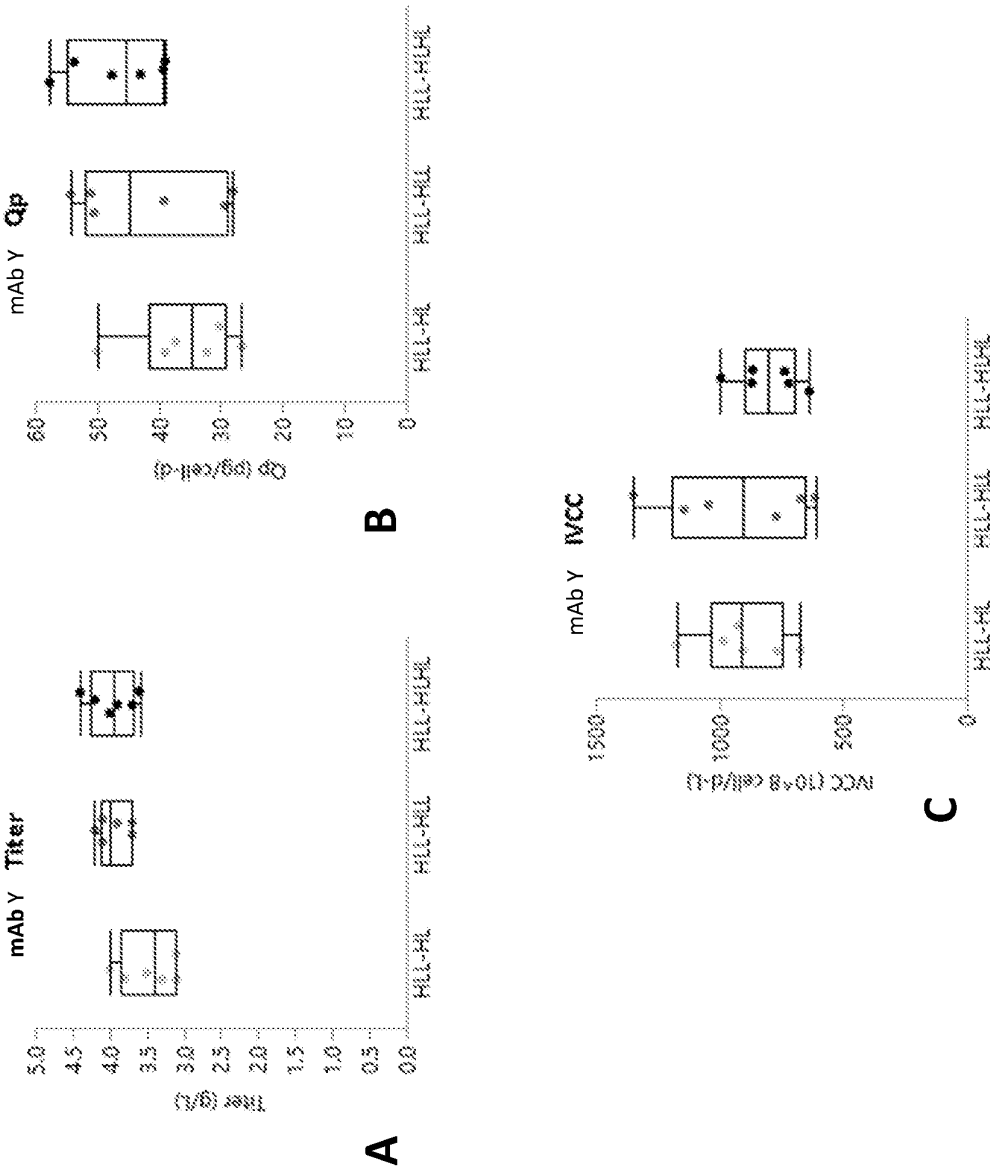

A
mAb Y Titer
Titer (g/L)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
HLL-HL
HLL-HLL
HLL-HLHL
B
mAb Y Qp
Qp (pg/cell-d)
60
50
40
30
20
10
0
HLL-HL
HLL-HLL
HLL-HLHL
C
mAb Y IVCC
IVCC (10^9 cell/d-L)
1500
1000
500
0
HLL-HL
HLL-HLL
HLL-HLHL

Figure 20.

Figure 20 (continued).

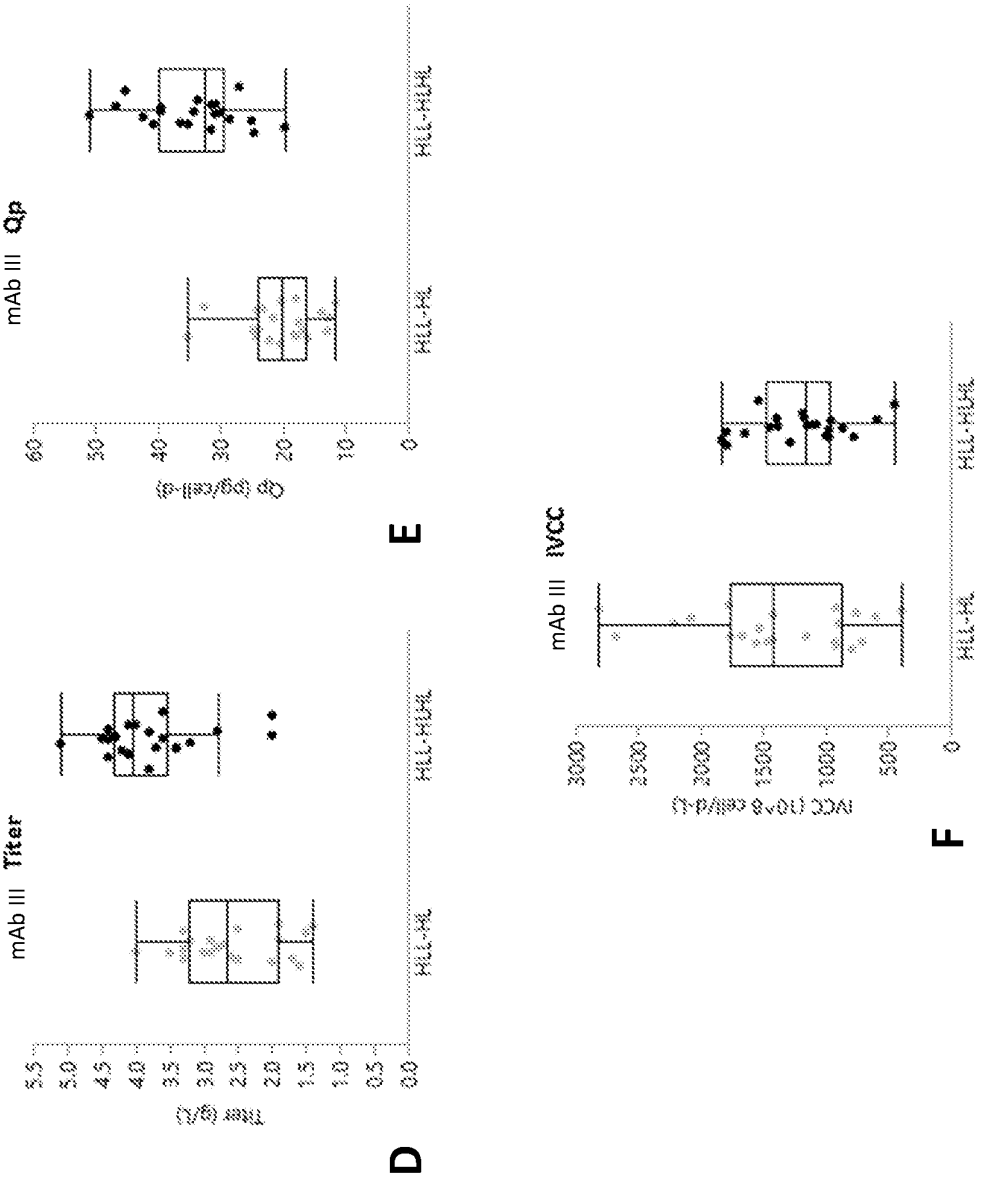
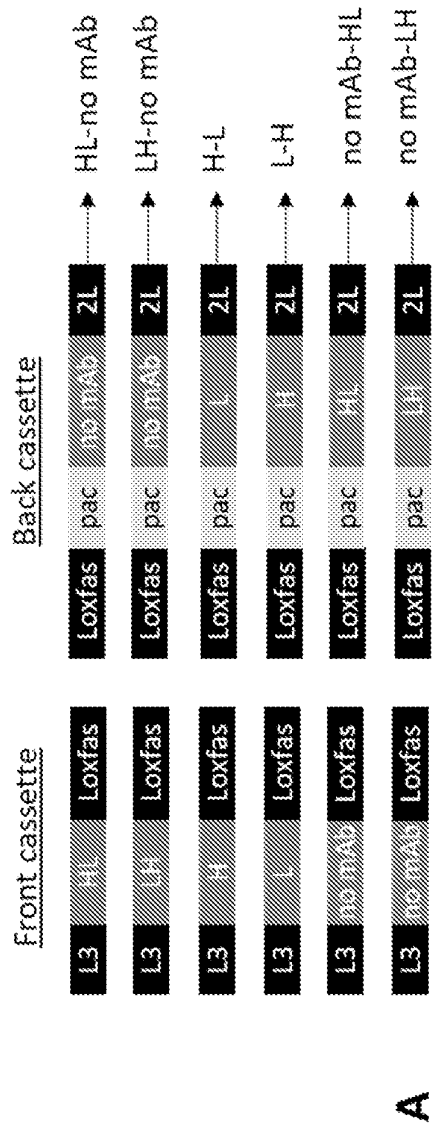


Figure 21.


Front cassette
Back cassette
L3
HL
LH
H
L
no mAb
Loxfas
pac
2L
HL-no mAb
LH-no mAb
H-L
L-H
no mAb-HL
no mAb-LH
A

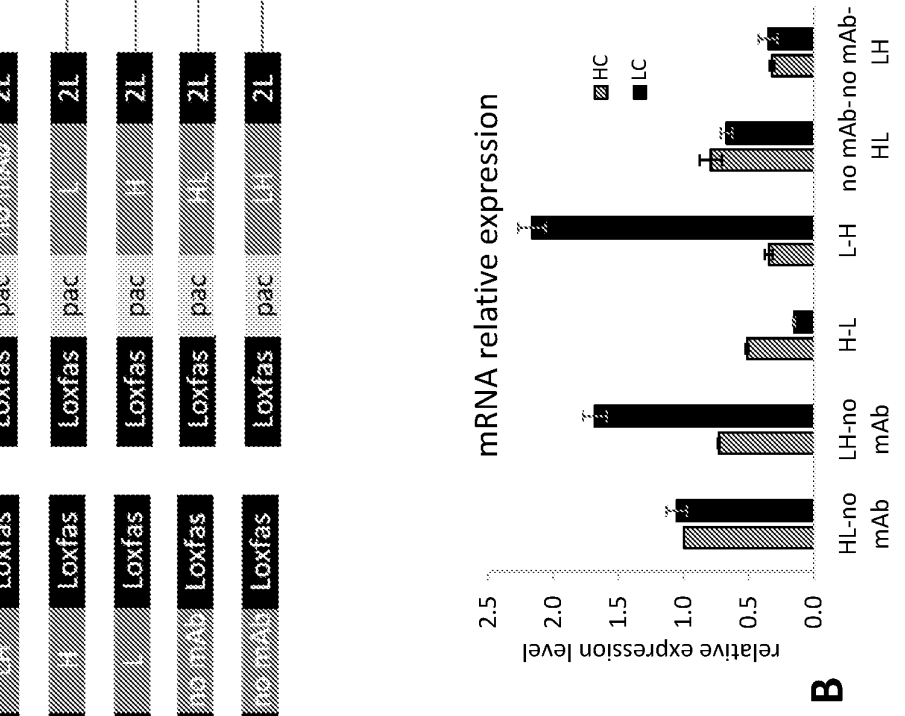
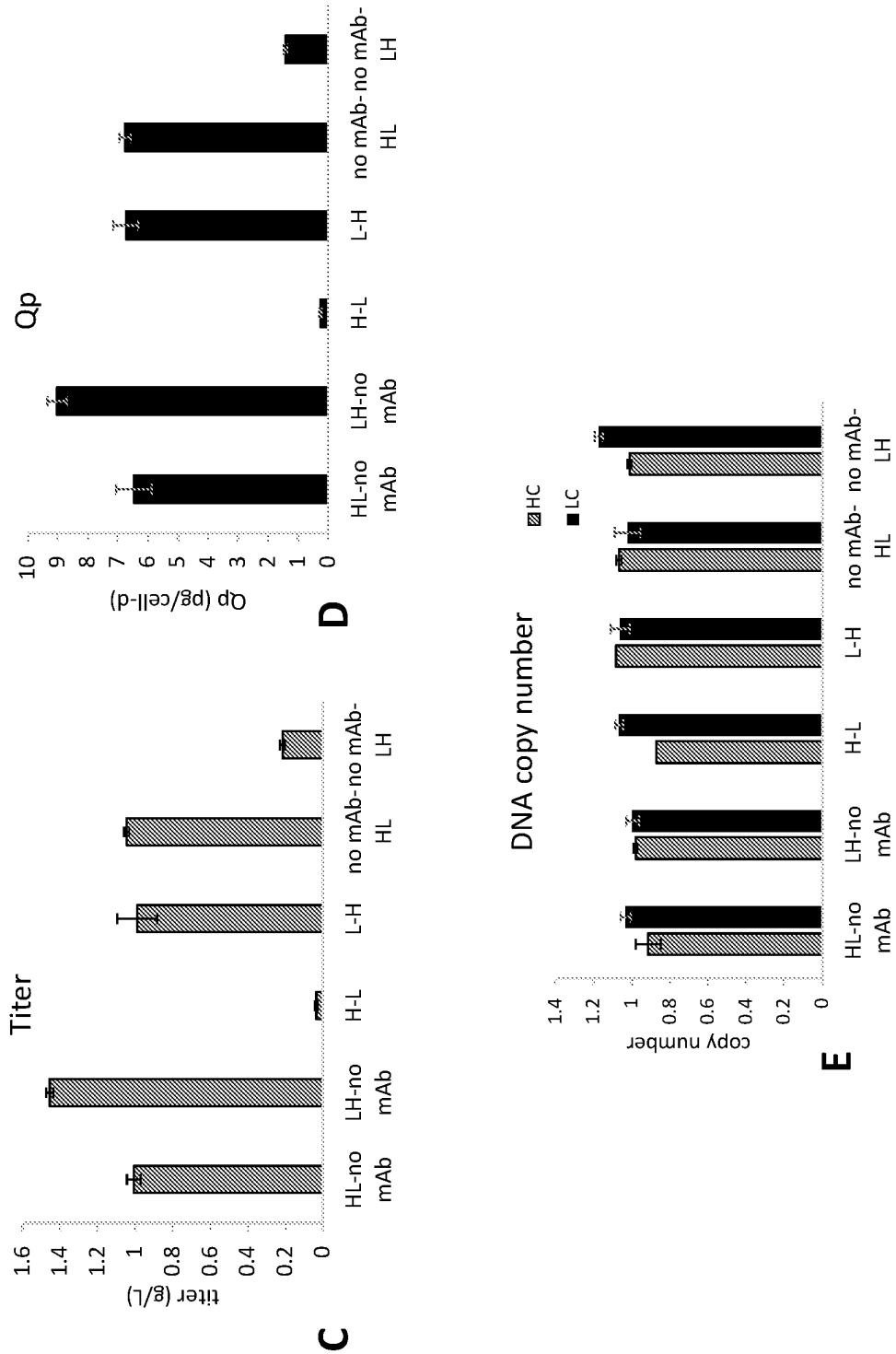

mRNA relative expression
relative expression level
2.5
2.0
1.5
1.0
0.5
0.0
HC
LC
HL-no mAb
LH-no mAb
H-L
L-H
no mAb-HL
no mAb-LH
B

Figure 21 (continued).



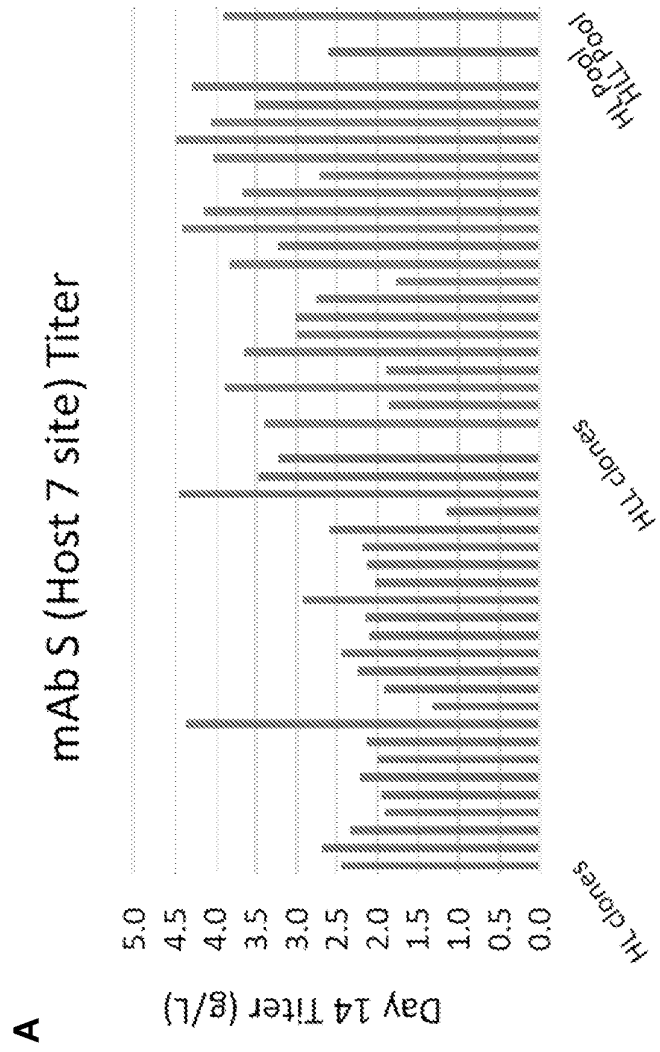

A
mAb S (Host 7 site) Titer
Day 14 Titer (g/L)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
HL clones
HLL clones
HL Pool
HLL Pool
Plasmid configuration
HC: LC ratio: 1:1= HL
1:2 = HLL

Figure 22.

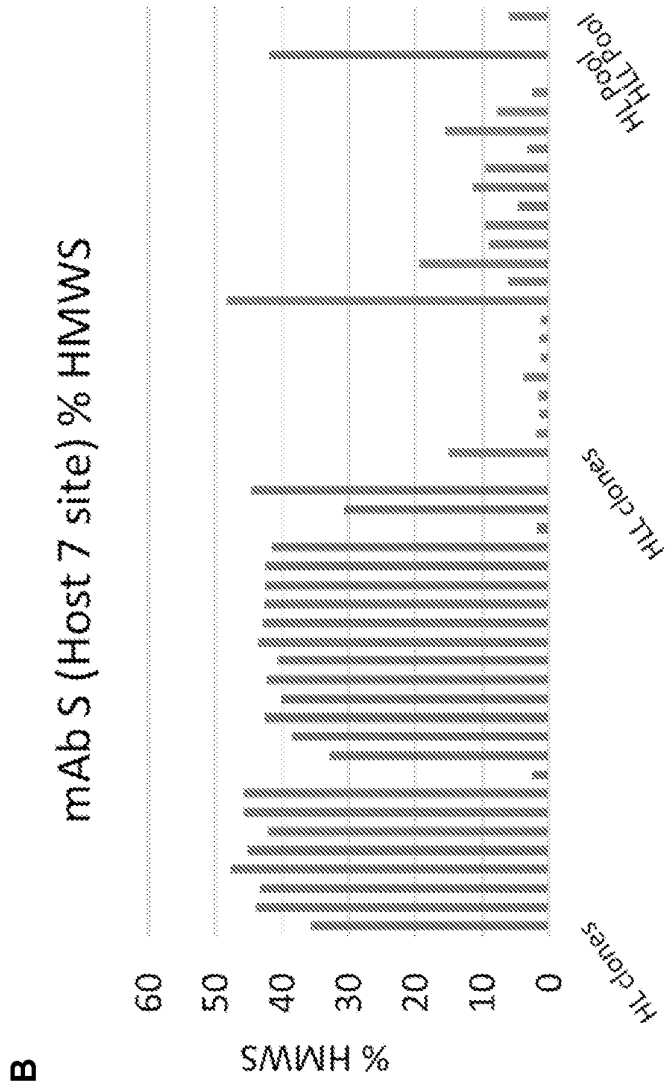


Figure 22 (continue).

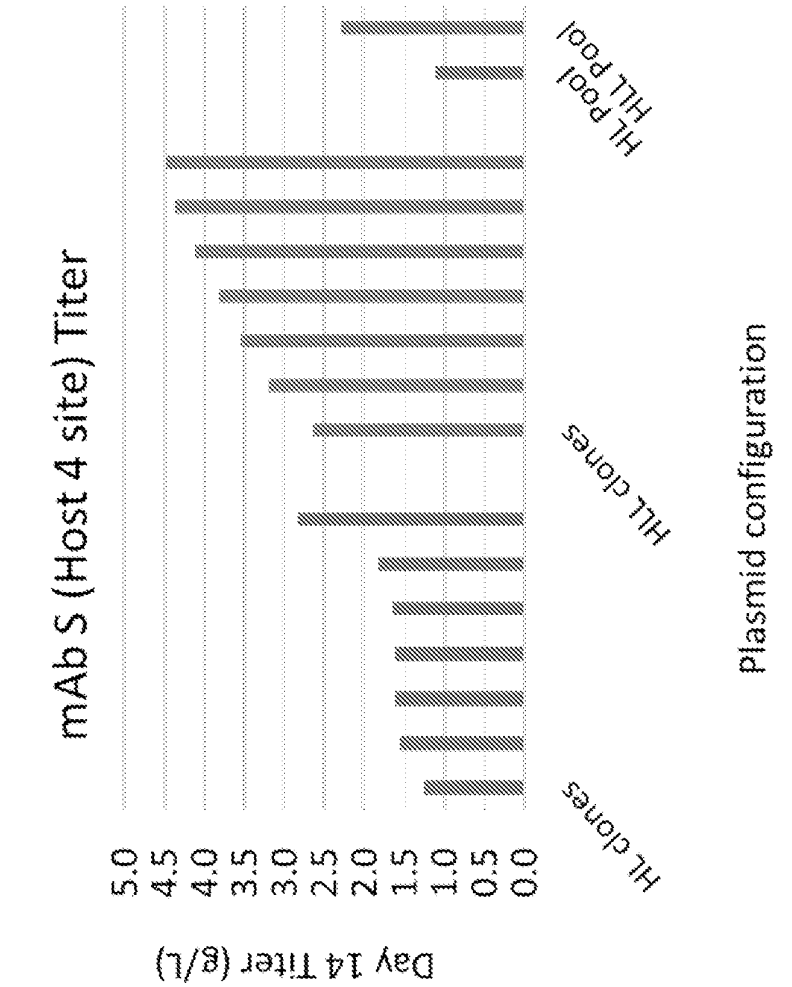


Figure 22 (continue).

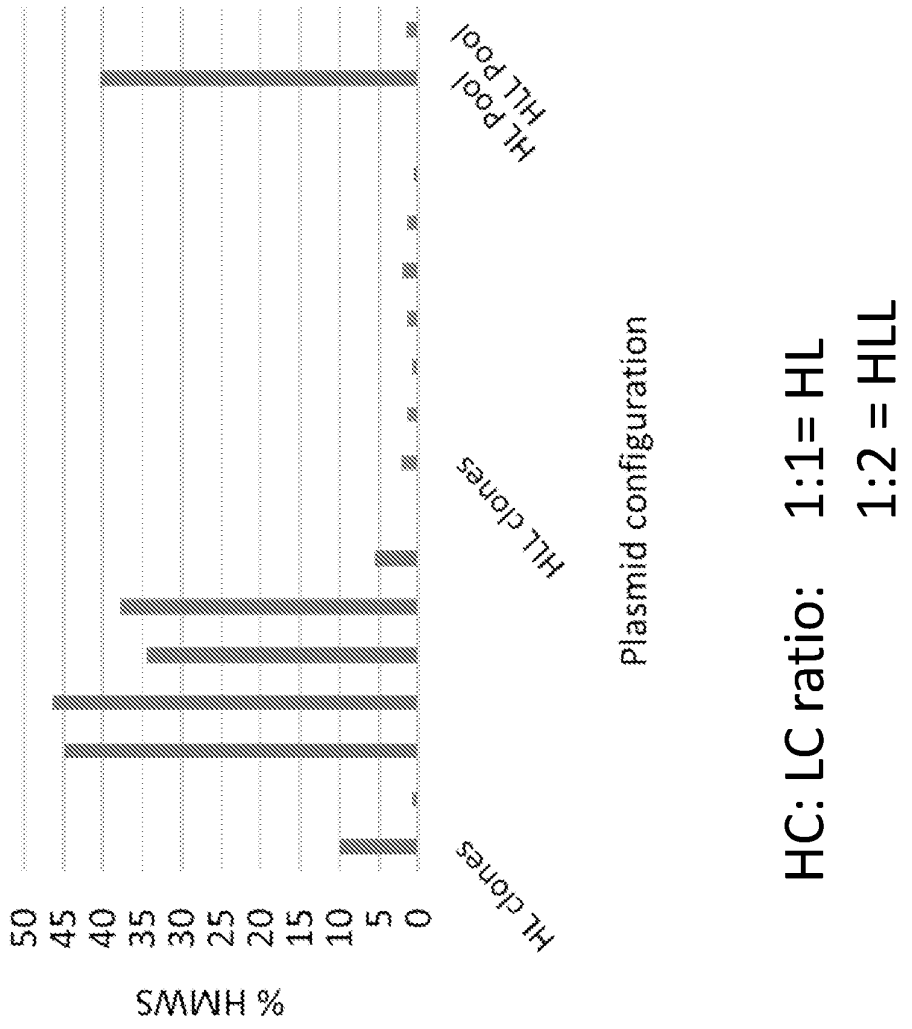


Figure 22 (continue).

Figure 23.

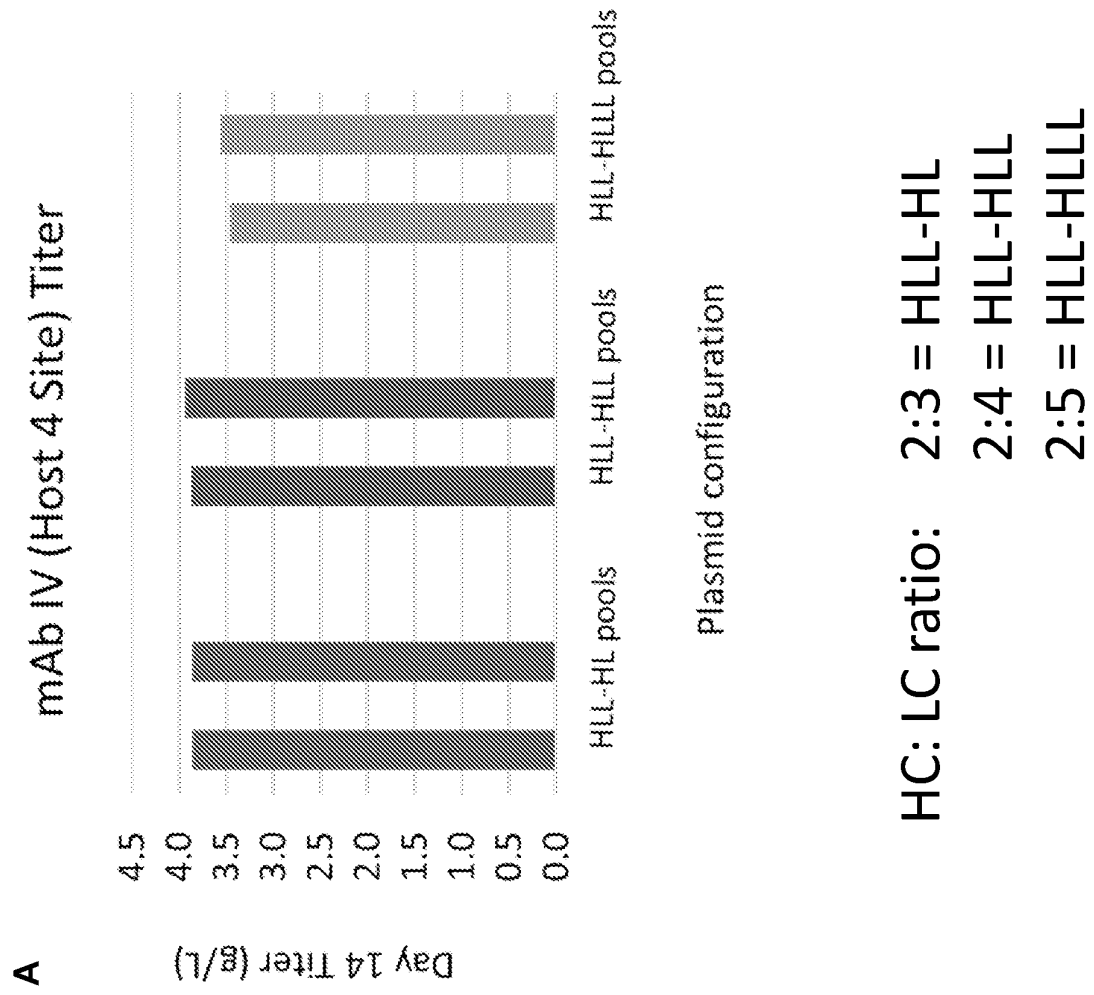
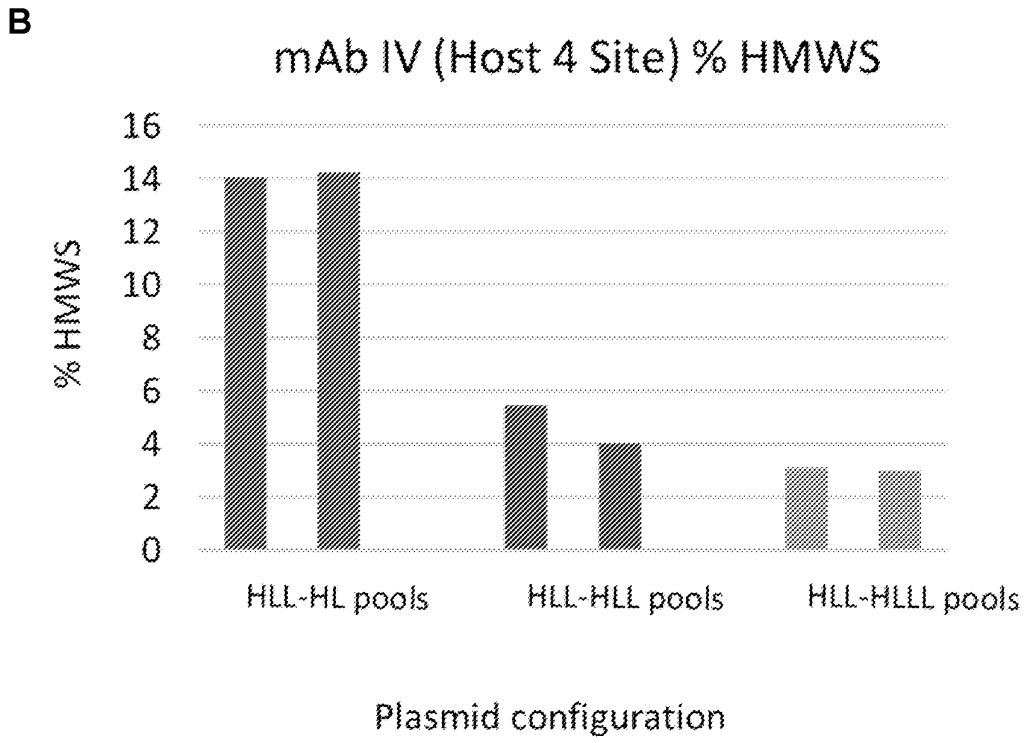

A
mAb IV (Host 4 Site) Titer
Day 14 Titer (g/L)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
HLL-HL pools
HLL-HLL pools
HLL-HLLL pools
Plasmid configuration
HC: LC ratio: 2:3 = HLL-HL
2:4 = HLL-HLL
2:5 = HLL-HLLL

Figure 23 (continue).



HC: LC ratio: 2:3 = HLL-HL
2:4 = HLL-HLL
2:5 = HLL-HLLL

Figure 24.

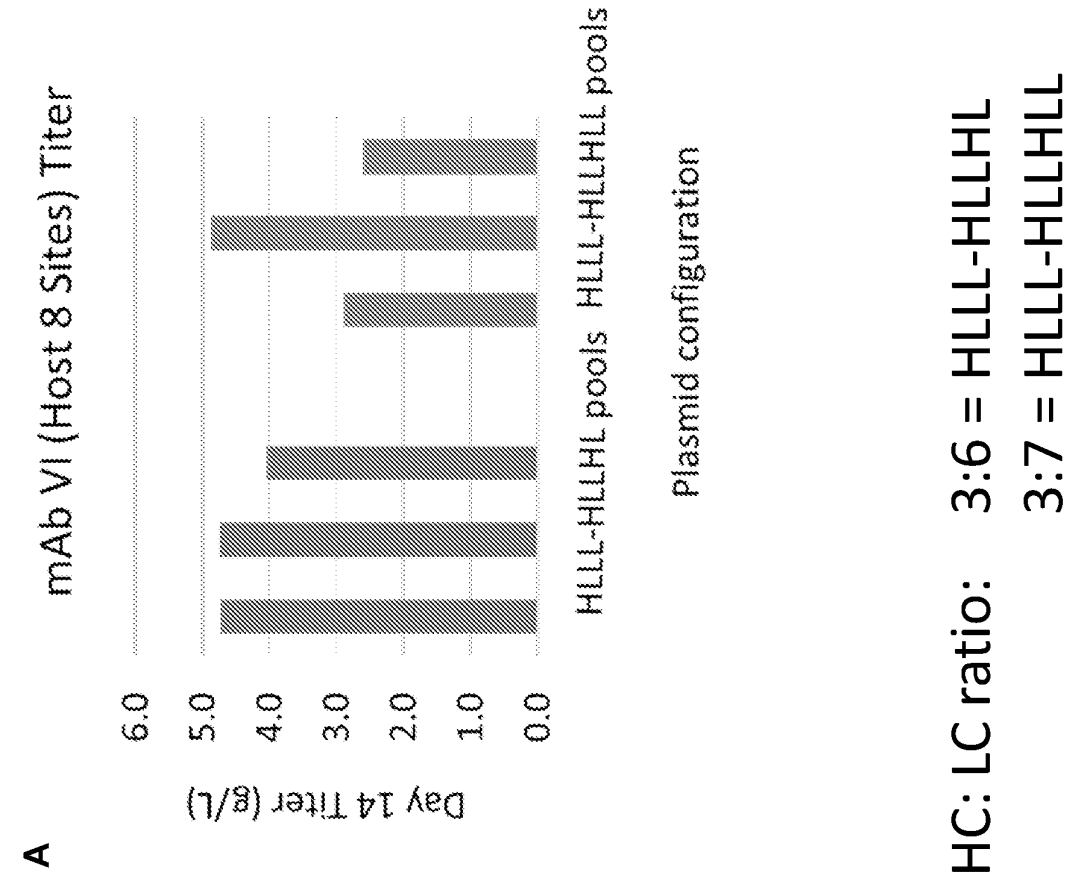
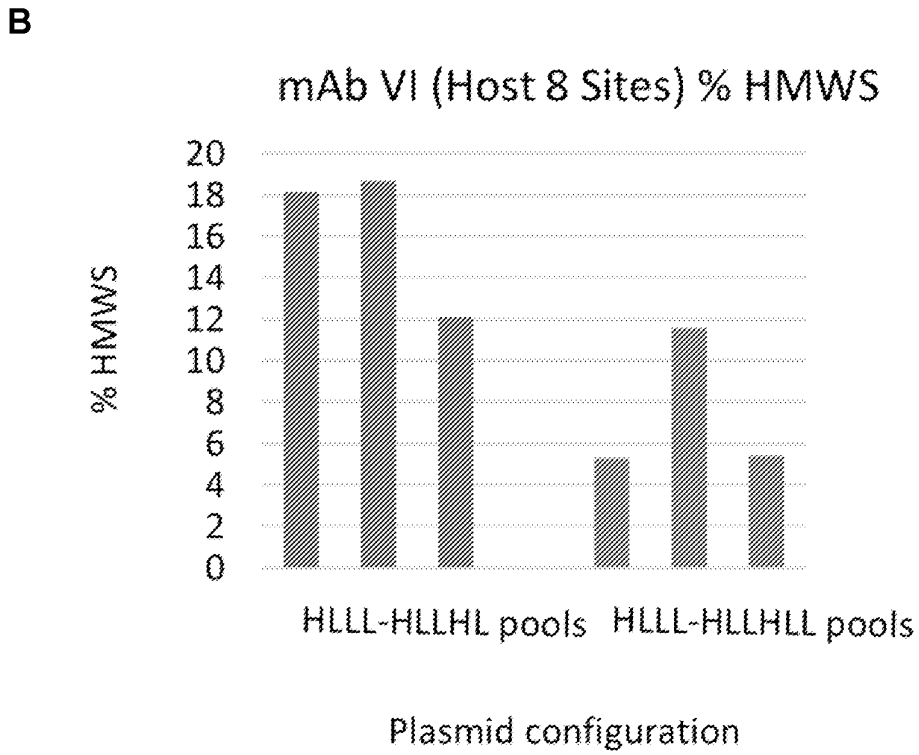

A
mAb VI (Host 8 Sites) Titer
Day 14 Titer (g/L)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
HLLL-HLLHL pools
HLLL-HLLHLL pools
Plasmid configuration
HC: LC ratio: 3:6 = HLLL-HLLHL
3:7 = HLLL-HLLHLL

Figure 24 (continue).



HC: LC ratio: 3:6 = HLLL-HLLHL
3:7 = HLLL-HLLHLL

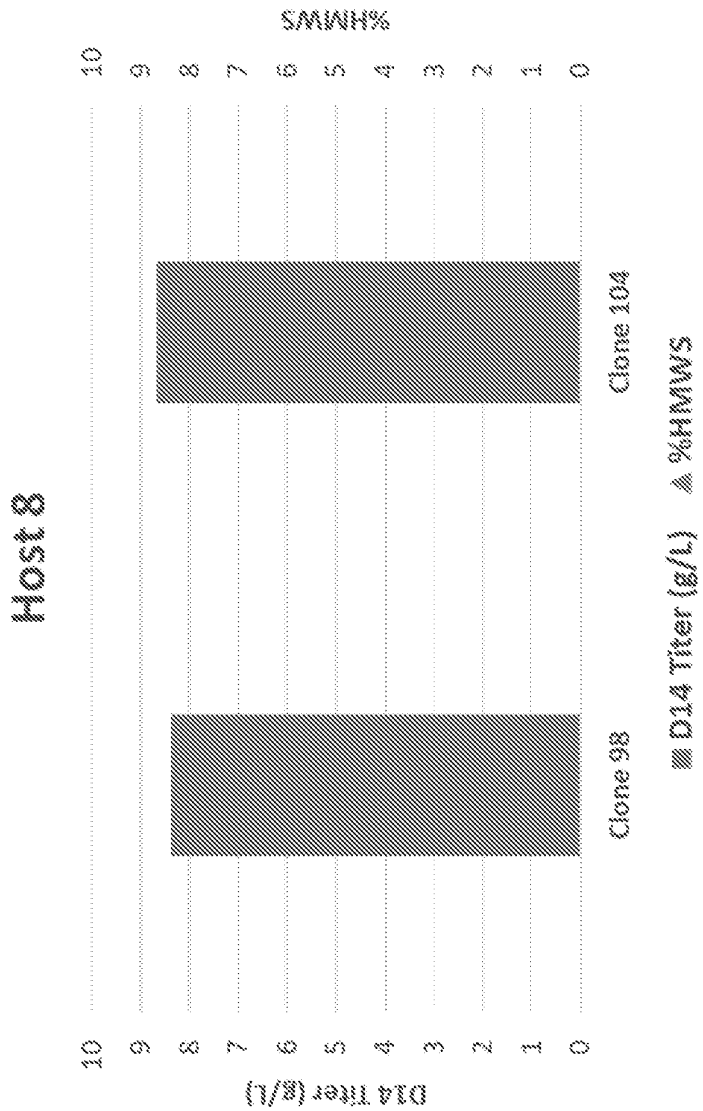

Host 8
D14 Titer (g/L)
%HMWS
0
1
2
3
4
5
6
7
8
9
10
Clone 98
Clone 104
D14 Titer (g/L)
%HMWS

Figure 25.

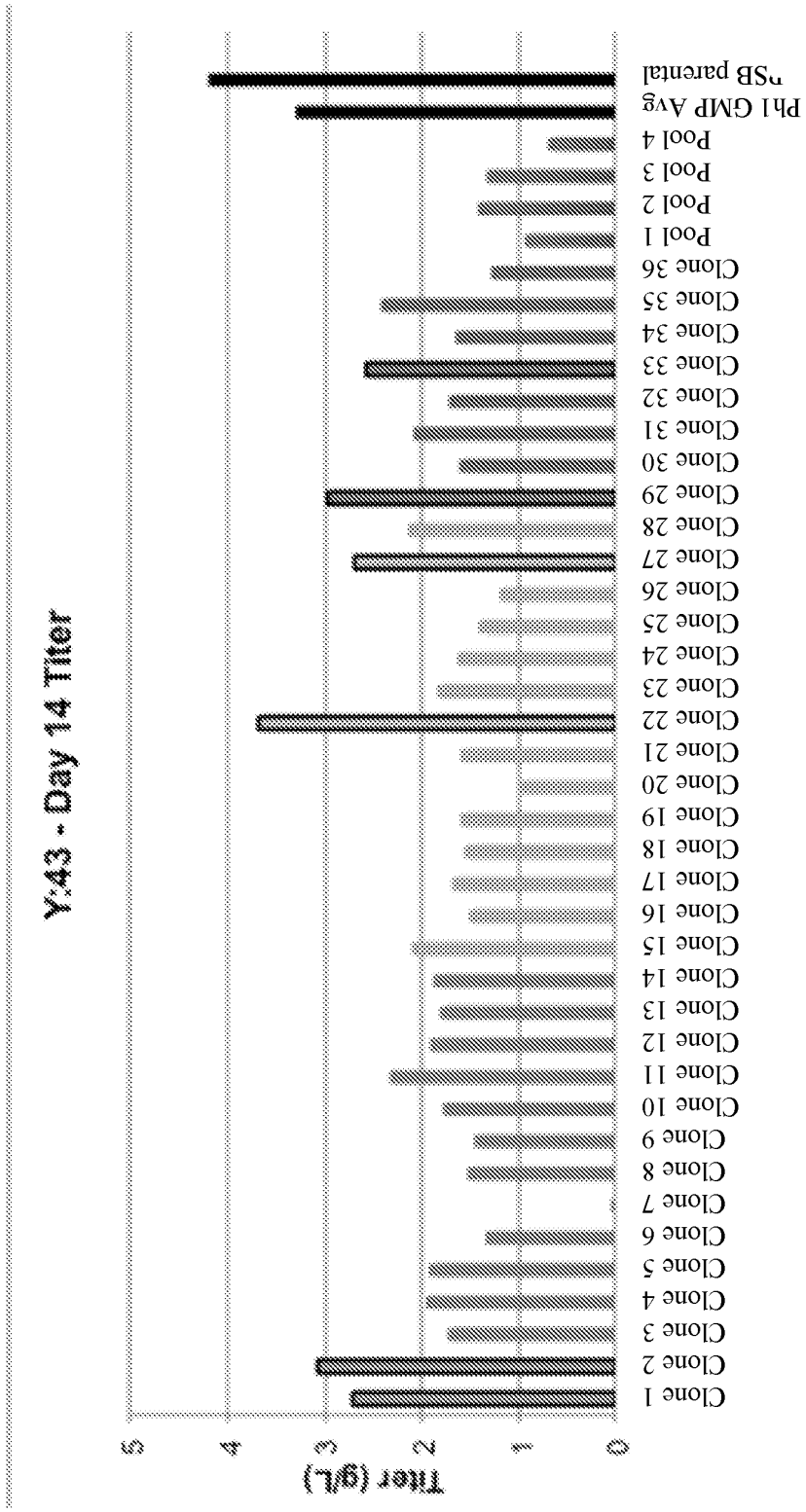

Y:43 - Day 14 Titer
Titer (g/L)
0
1
2
3
4
5
Clone 1
Clone 2
Clone 3
Clone 4
Clone 5
Clone 6
Clone 7
Clone 8
Clone 9
Clone 10
Clone 11
Clone 12
Clone 13
Clone 14
Clone 15
Clone 16
Clone 17
Clone 18
Clone 19
Clone 20
Clone 21
Clone 22
Clone 23
Clone 24
Clone 25
Clone 26
Clone 27
Clone 28
Clone 29
Clone 30
Clone 31
Clone 32
Clone 33
Clone 34
Clone 35
Clone 36
Pool 1
Pool 2
Pool 3
Pool 4
Ph1 GMP Avg
nSB parental

Figure 26.

Figure 27.

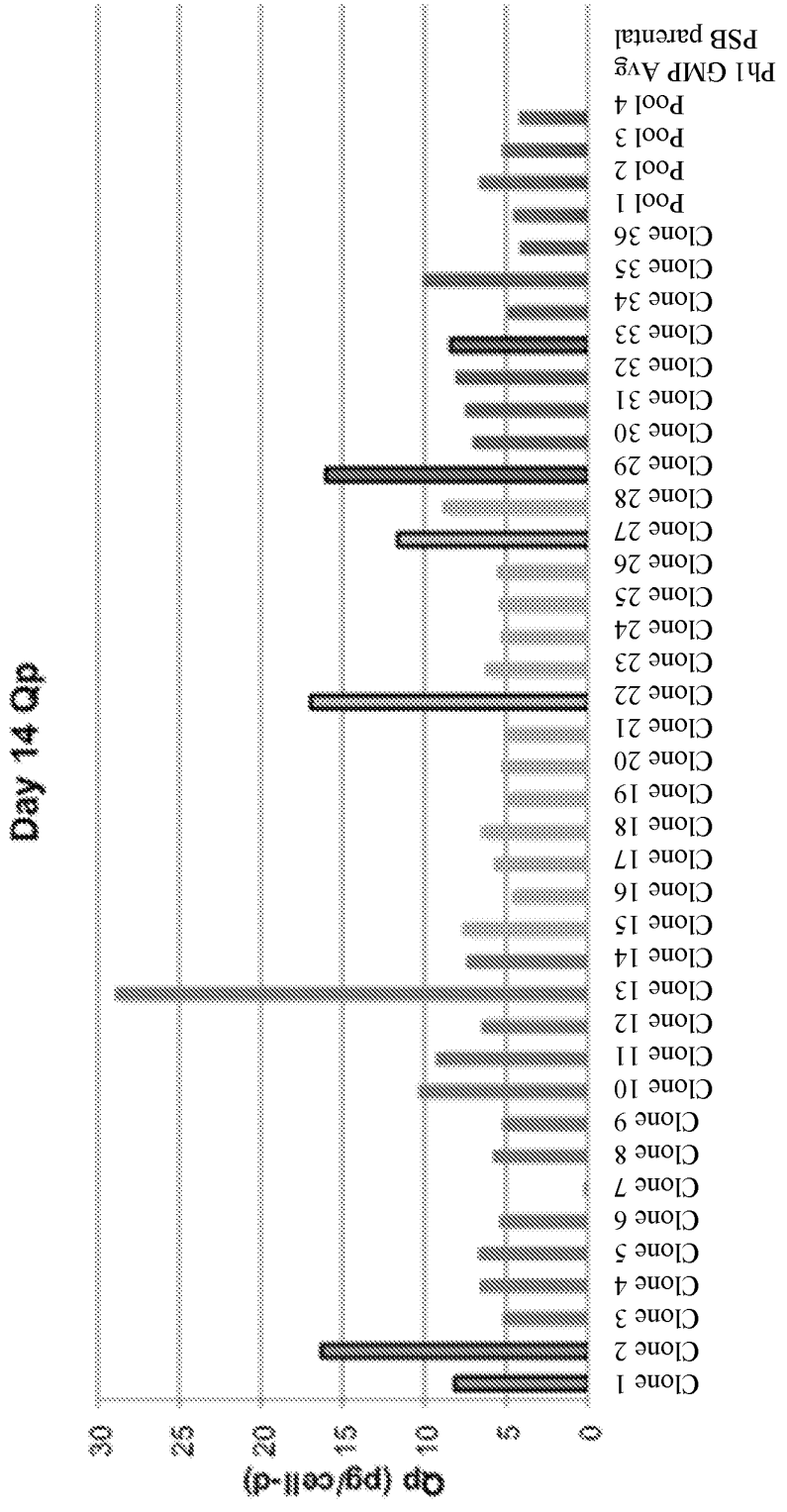

Day 14 Qp
Qp (pg/cell-d)
0
5
10
15
20
25
30
Clone 1
Clone 2
Clone 3
Clone 4
Clone 5
Clone 6
Clone 7
Clone 8
Clone 9
Clone 10
Clone 11
Clone 12
Clone 13
Clone 14
Clone 15
Clone 16
Clone 17
Clone 18
Clone 19
Clone 20
Clone 21
Clone 22
Clone 23
Clone 24
Clone 25
Clone 26
Clone 27
Clone 28
Clone 29
Clone 30
Clone 31
Clone 32
Clone 33
Clone 34
Clone 35
Clone 36
Pool 1
Pool 2
Pool 3
Pool 4
Ph1 GMP Avg
PSB parental

Figure 28.

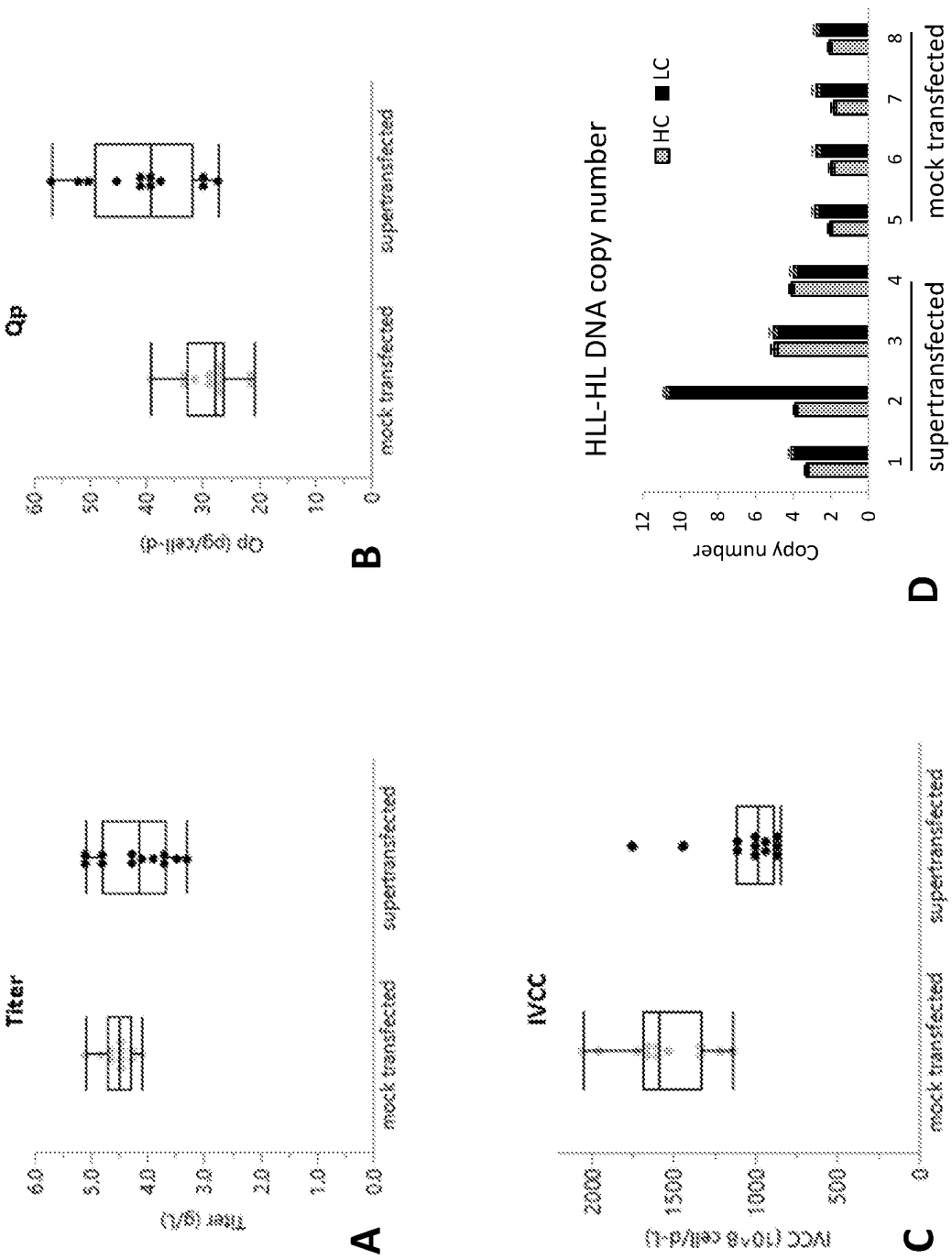
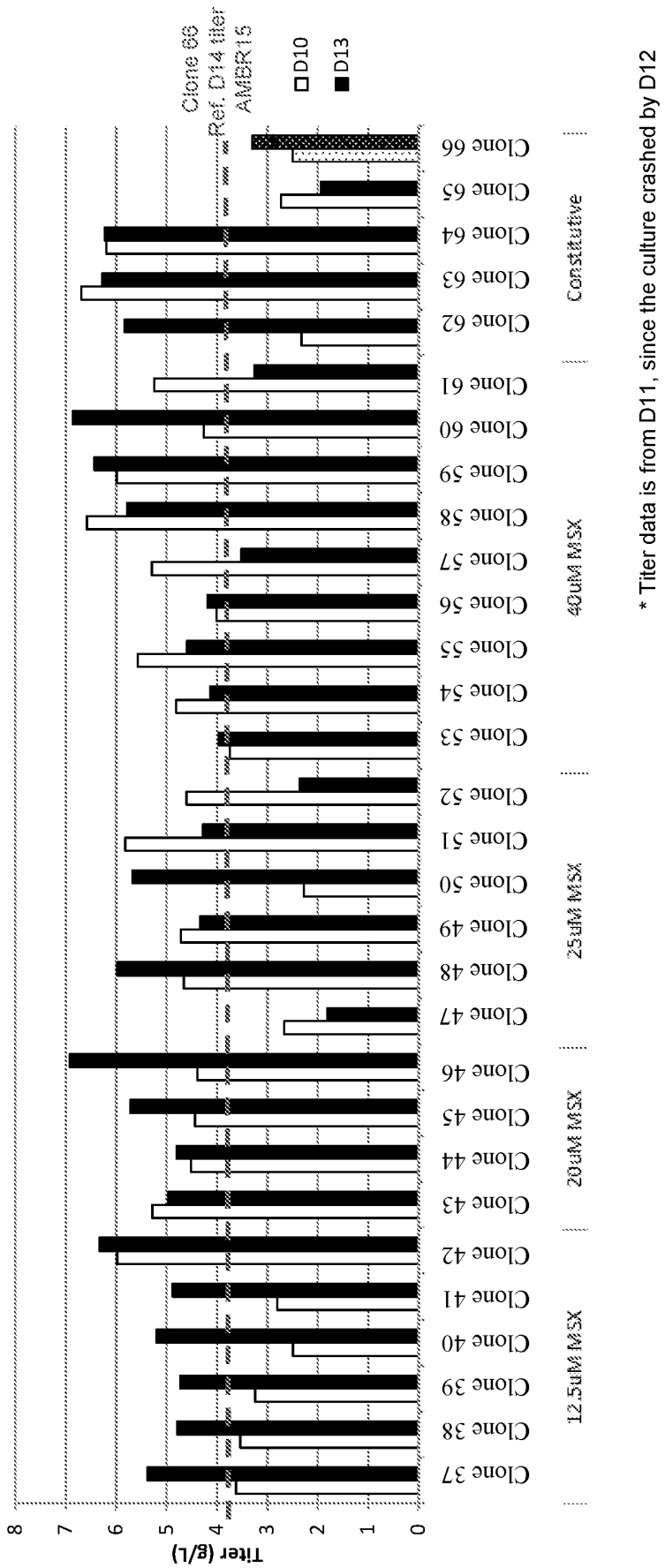

Titer
Titer (g/L)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
mock transfected
supertransfected
A
Qp
Qp (pg/cell-d)
60
50
40
30
20
10
0
mock transfected
supertransfected
B
IVCC
IVCC (10^6 cell/d-L)
2000
1500
1000
500
0
mock transfected
supertransfected
C
HLL-HL DNA copy number
HC
LC
Copy number
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
supertransfected
mock transfected
D

Figure 29.

A



* Titer data is from D11, since the culture crashed by D12

Figure 29 (continue).

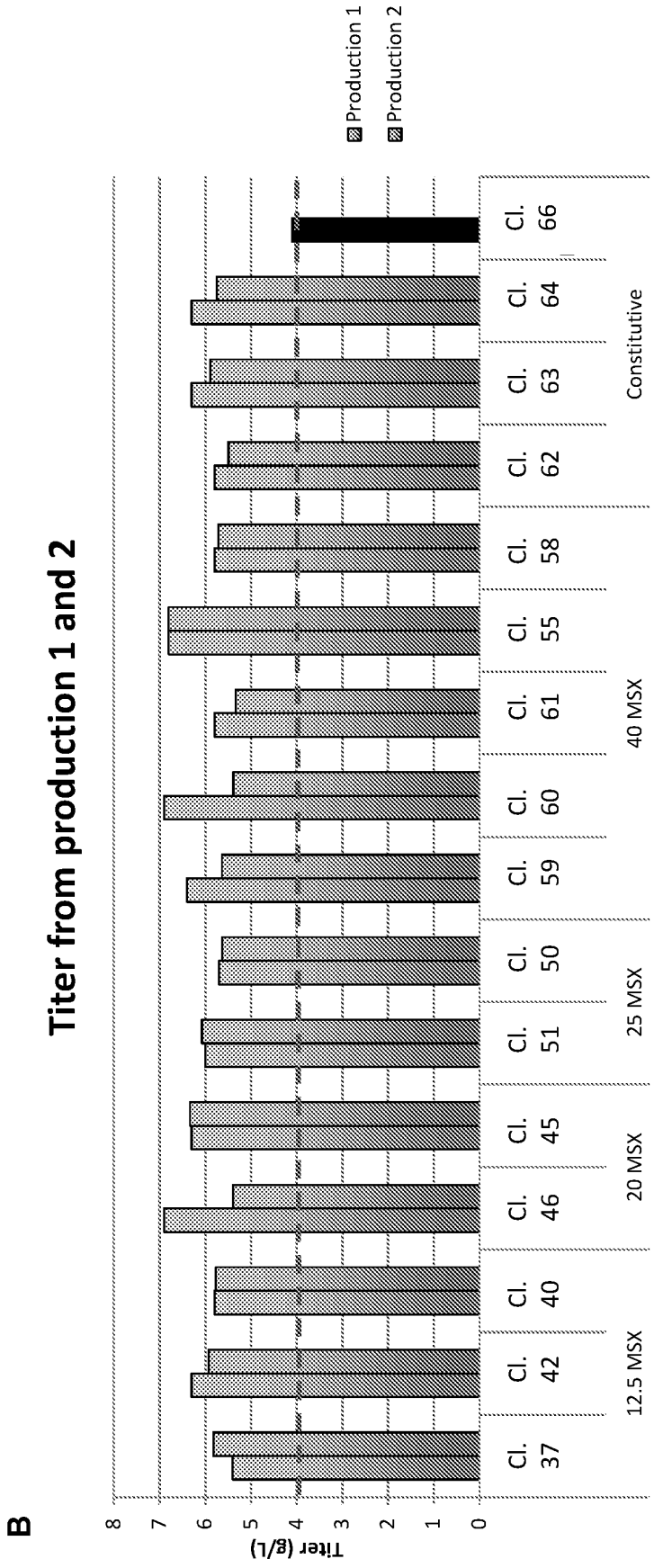


Figure 29 (continue).

