

FIG. 1

Name	Sequence
Kz.Wt / consensus	ACC <u>A</u> IG <u>G</u>
Kz.G (A ₃ >G)	GCC <u>A</u> IG <u>G</u>
Kz.T (A ₃ >T)	TCC <u>A</u> IG <u>G</u>
Kz.TAG (ACC ₃ >TAG)	TAG <u>A</u> IG <u>G</u>
Kz.3T (ACC ₃ >TTT)	TTT <u>A</u> IG <u>G</u>

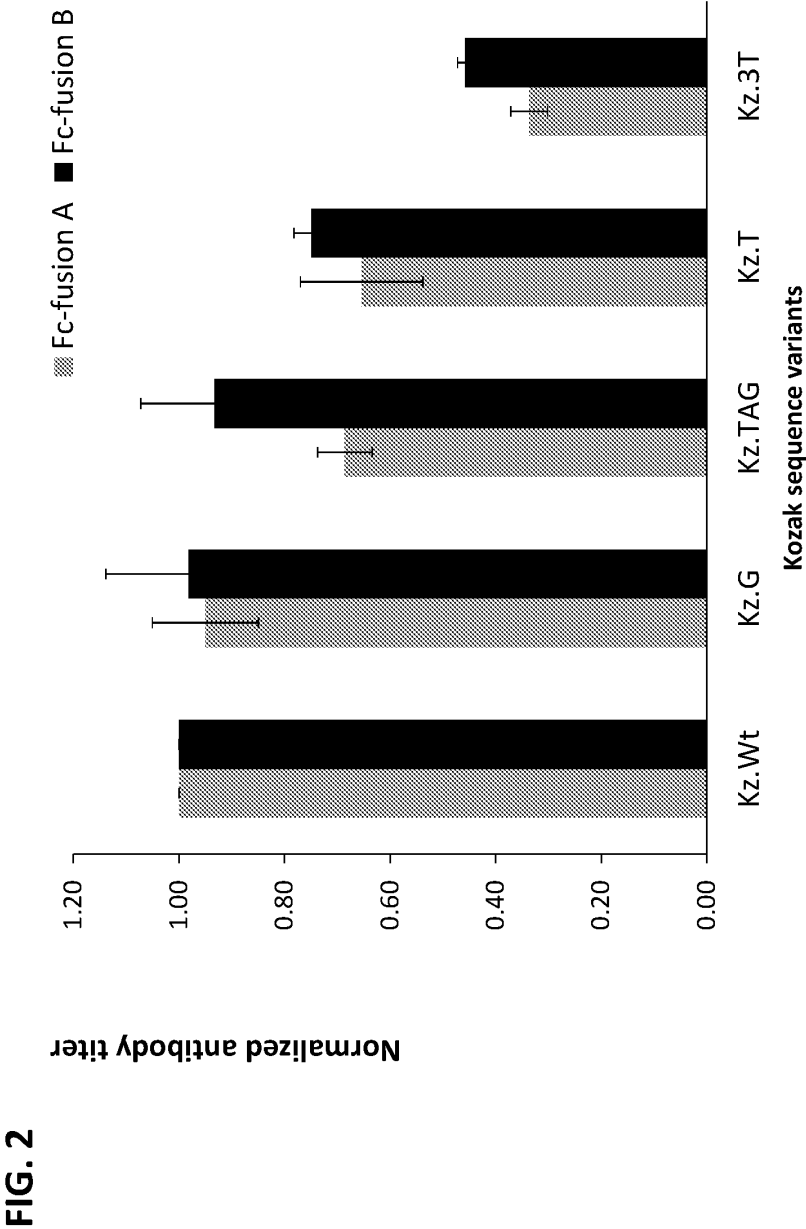


FIG. 3

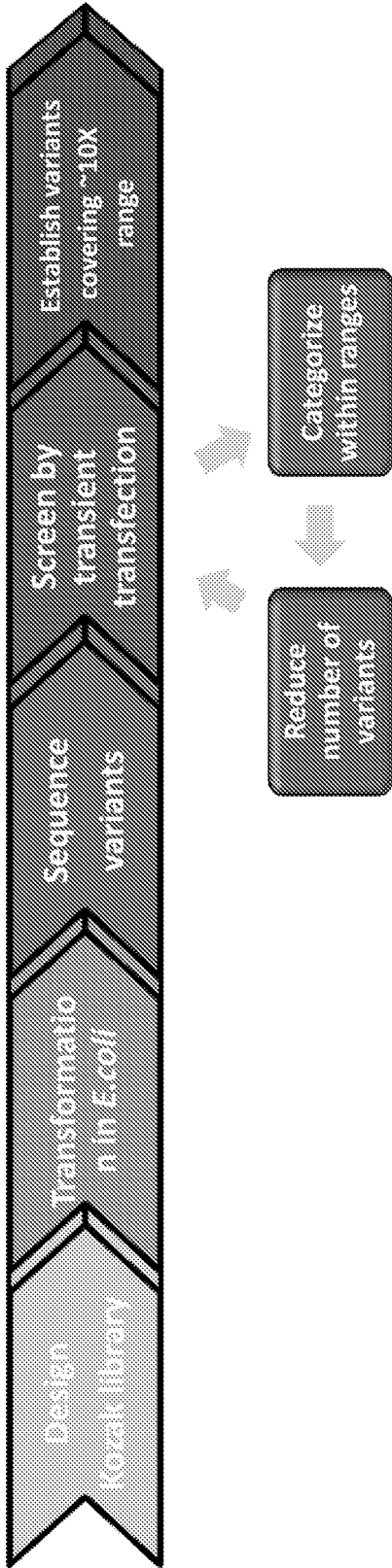


FIG. 4

Kozak Wt 5'-CCACCATG⁻³GGA⁺⁴-3'

Kozak library 5'-NNNNNATG⁻³NGA⁺⁴-3'

FIG. 5

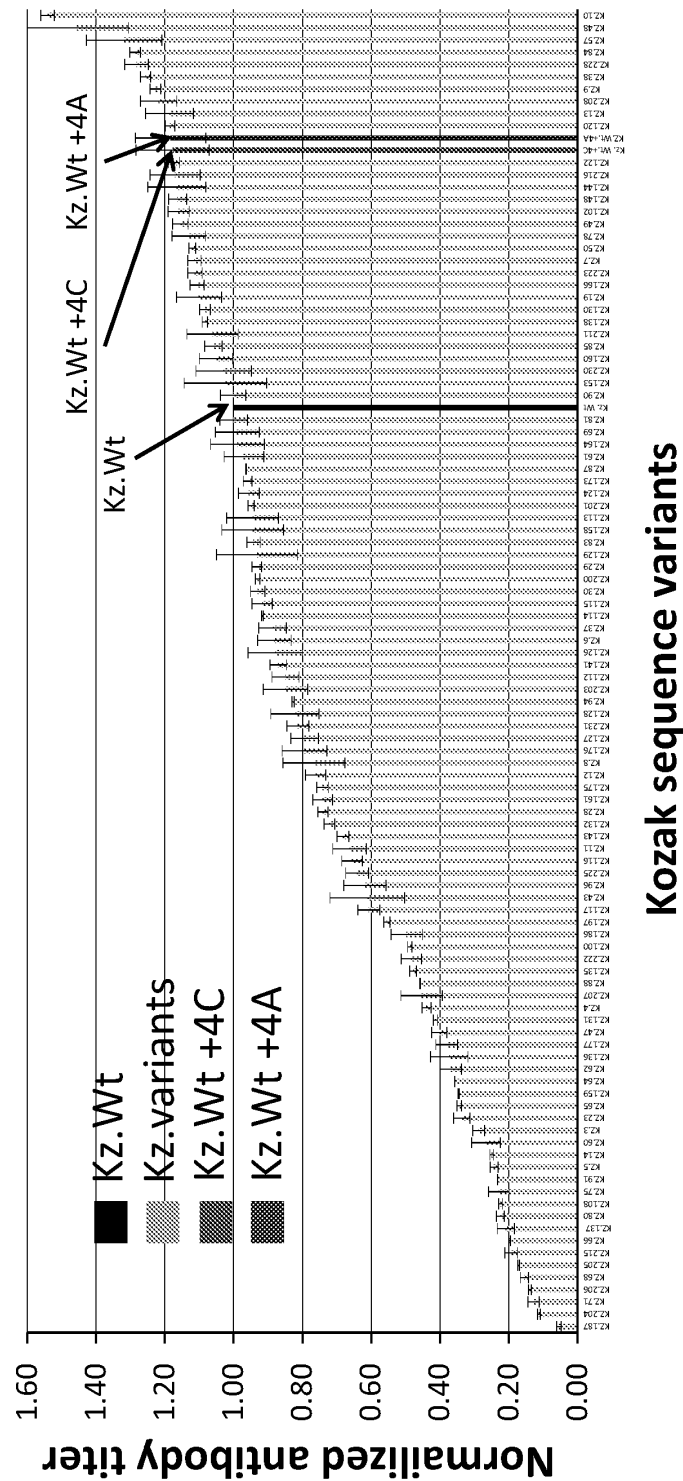


FIG. 6

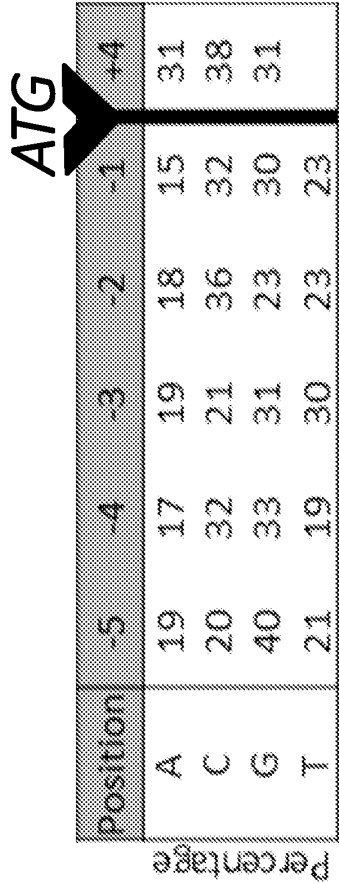


FIG. 7

Phase	Variants	Transient transfection (n times)*
1 st Round	108 variants	n=2
2 nd Round	66 variants	n=2
3 rd Round	28 variants	n=3
4 th Round	10 variants	n=4

* Different DNA prep

FIG. 8A

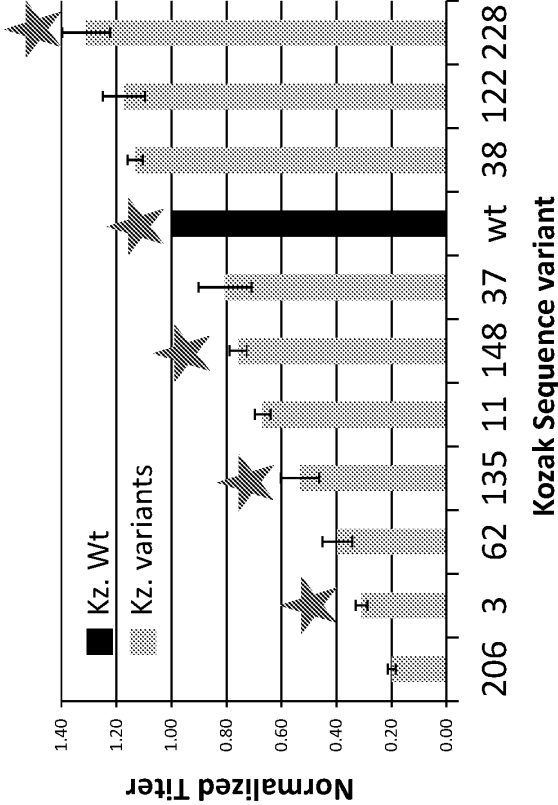


FIG. 8B

Variants	-5	-4	-3	-2	-1	+4
206	T	G	C	G	C	C
3	T	T	T	T	T	G
62	C	C	T	G	G	C
135	G	G	C	T	C	C
11	C	G	T	C	C	A
148	C	C	T	A	G	C
37	G	A	T	A	A	G
wt	C	C	A	C	C	G
38	C	G	A	C	C	C
122	G	C	G	A	C	C
228	G	A	A	G	T	A

FIG. 9

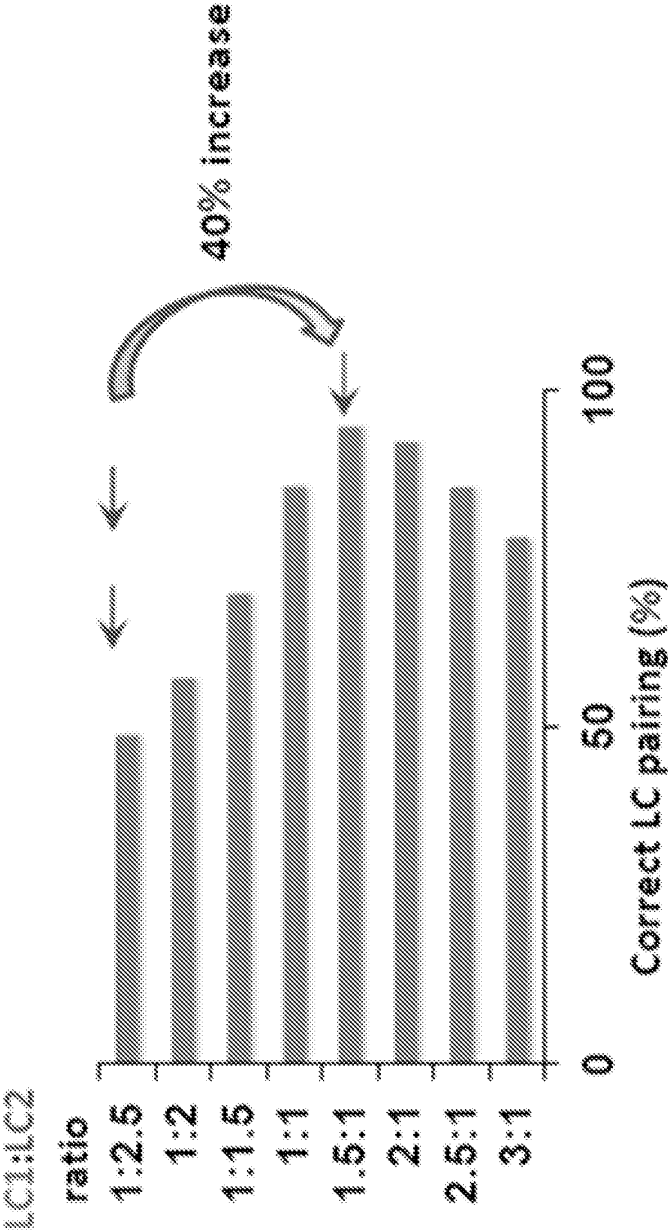


FIG. 10

Kozak variants selected		
Name	range	
Kz.3	(0.3x)	
Kz.135	(0.5x)	
Kz.148	(0.8x)	
Kz.Wt	(1x)	
Kz.228	(1.3x)	

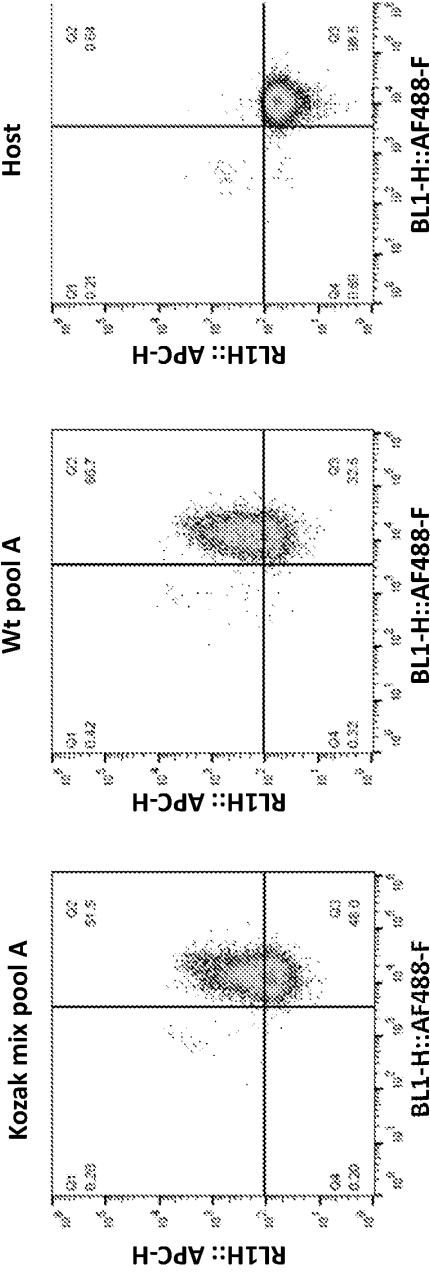
Name	Sequence
Kz.3	TTTTT ATG G
Kz.135	GGCTC ATG C
Kz.148	CCTAG ATG C
WT	CCACC ATG G
Kz.228	GAAGT ATG A

FIG. 11

Ab1		LC				
Front vectors		Kz.3	Kz.135	Kz.148	Kz.Wt	Kz.228
HC	Kz.3	1	2	3	4	5
	Kz.135	6	7	8	9	10
	K.148	11	12	13	14	15
	Kz.Wt	16	17	18	19	20
	Kz.228	21	22	23	24	25

FIG. 12

Transfection #1



Transfection #2

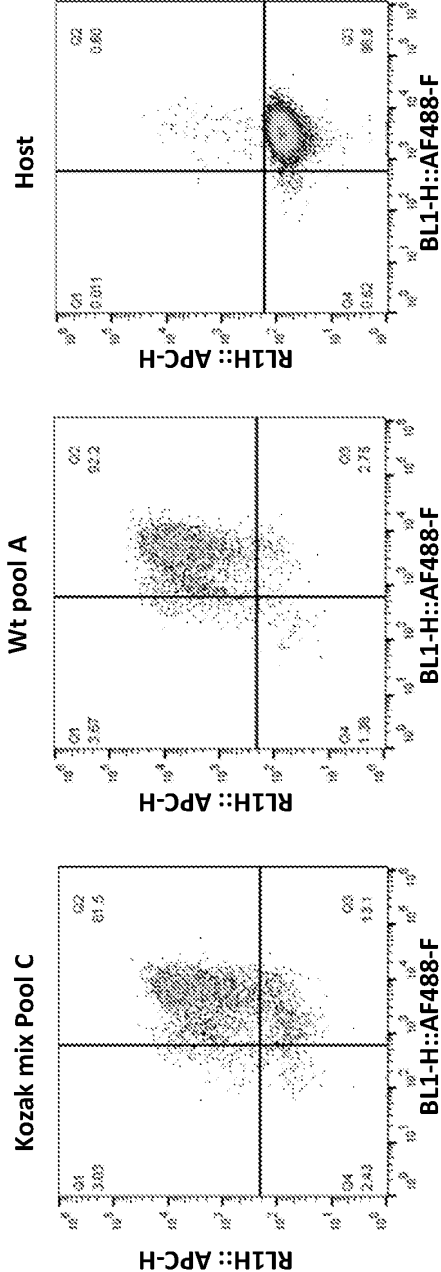


FIG. 13A

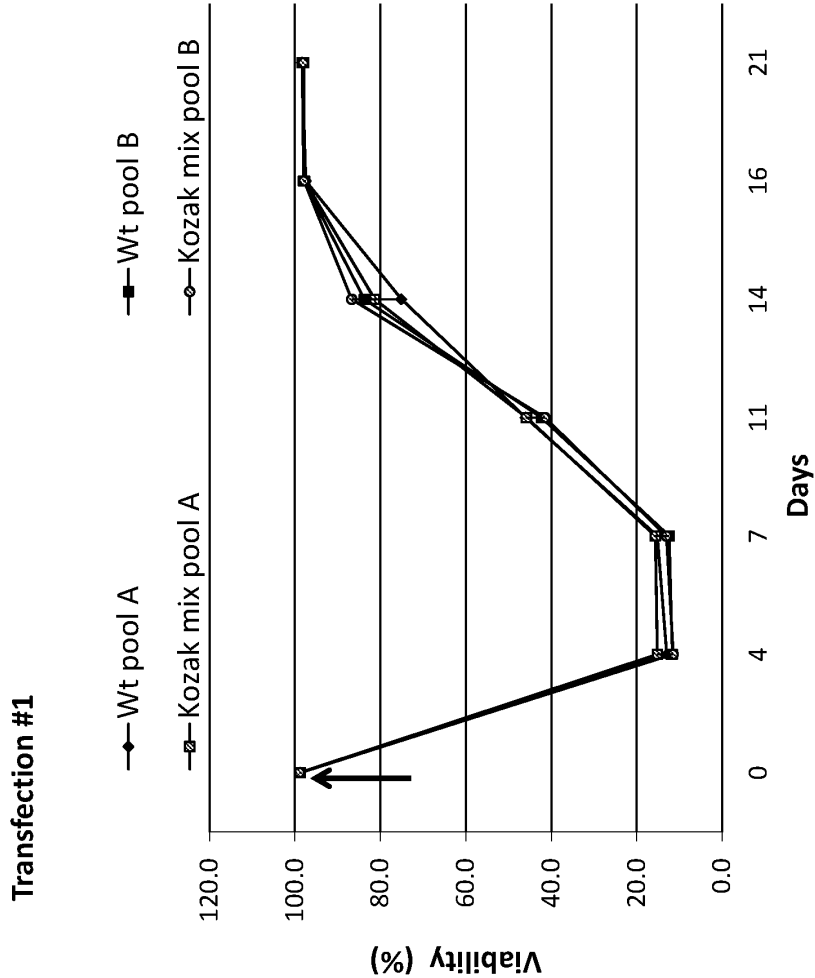


FIG. 13B

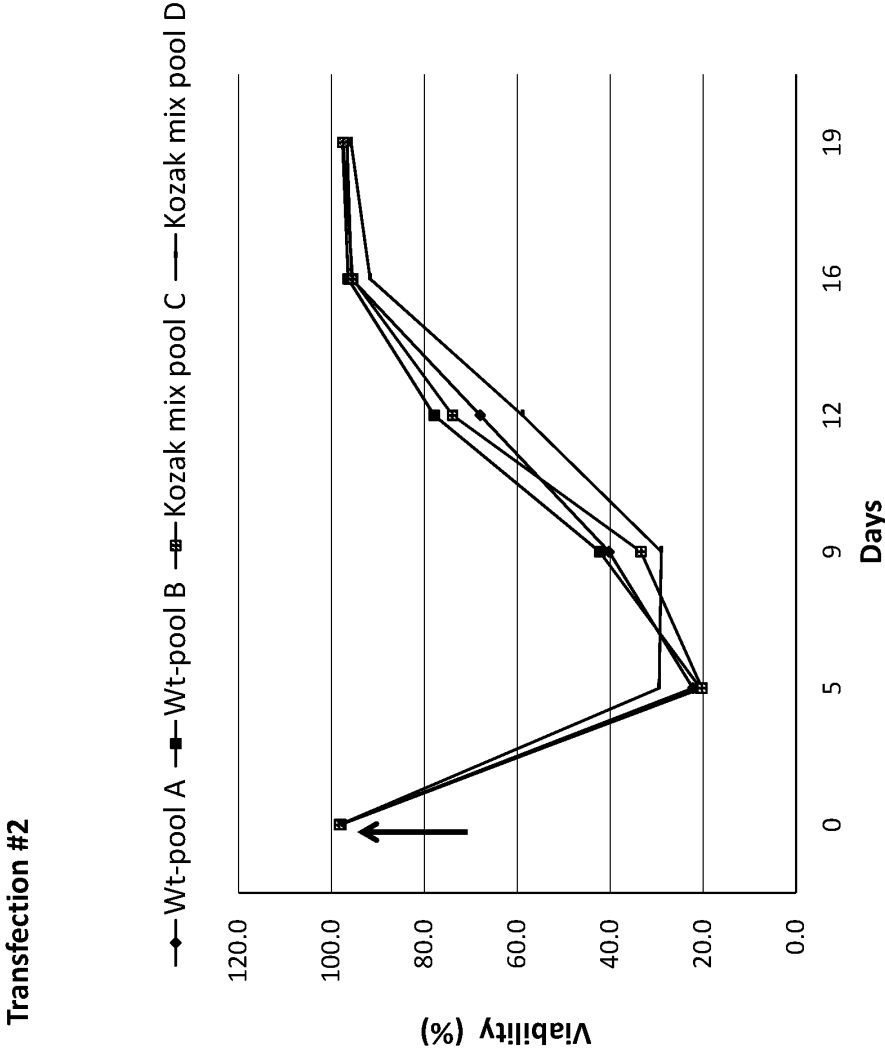


FIG. 14A

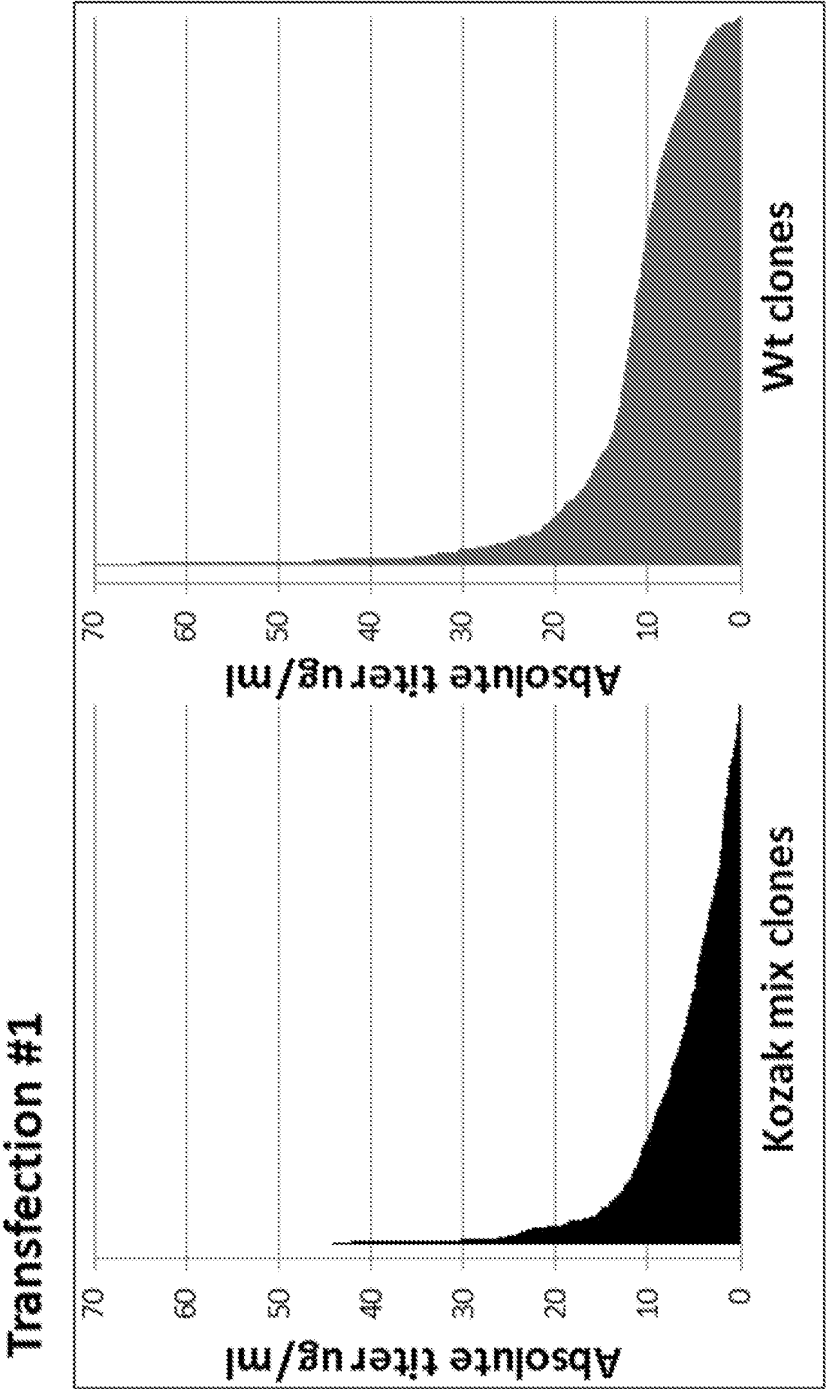


FIG. 14B

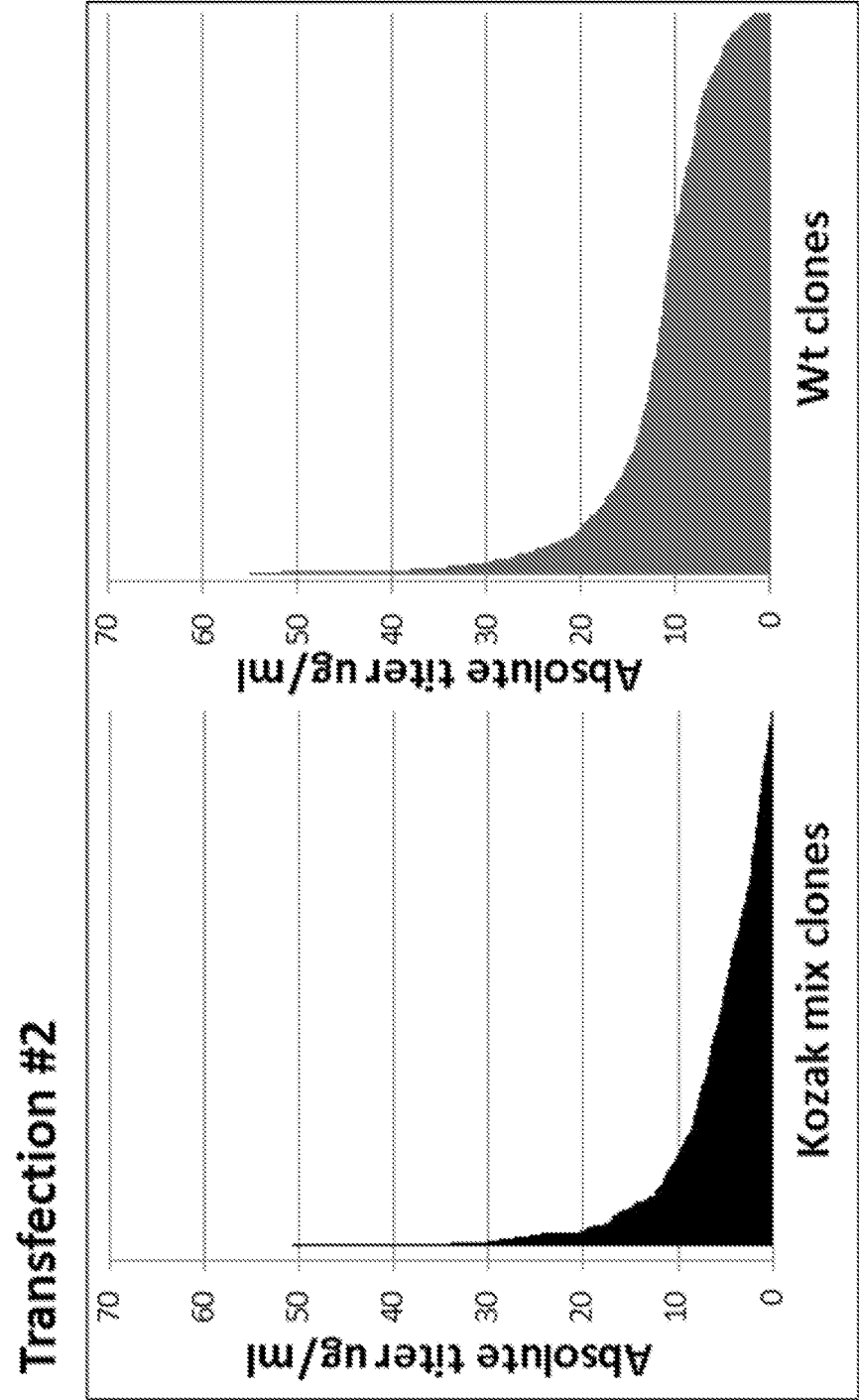


FIG. 15A

Transfection #1

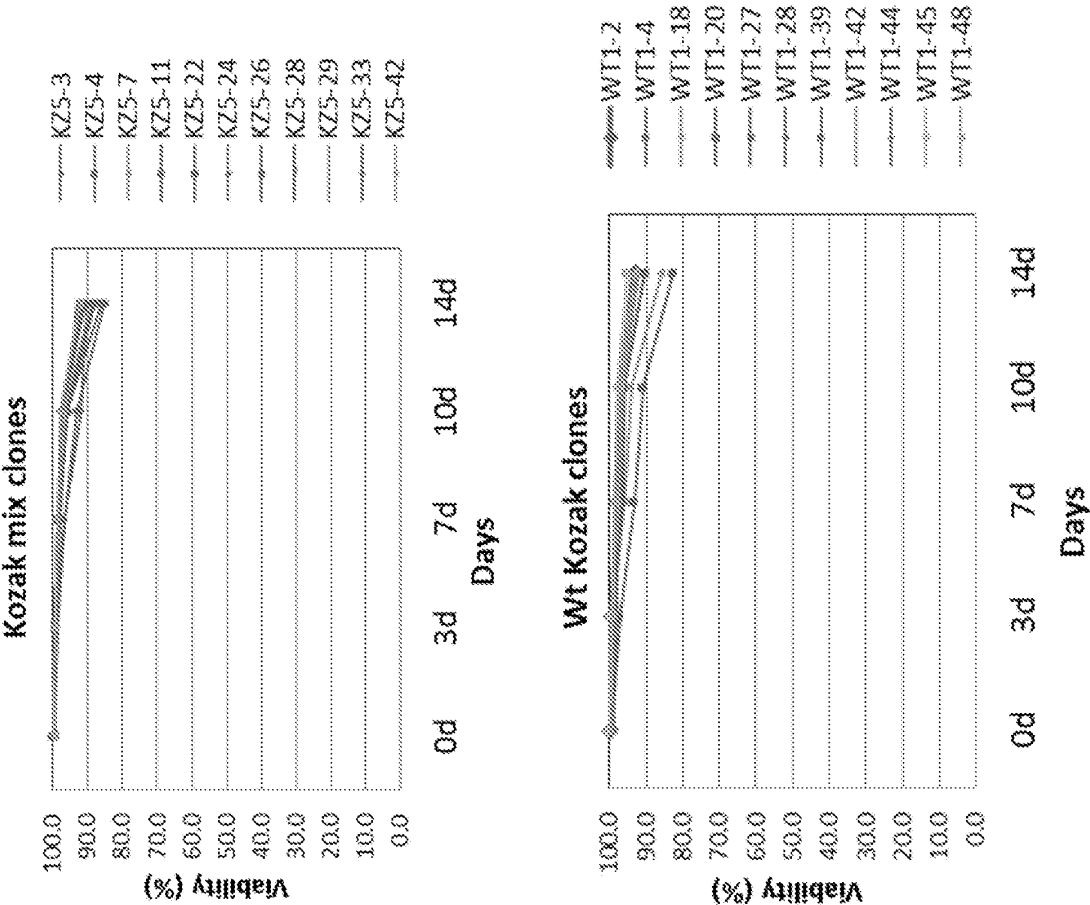


FIG. 15B

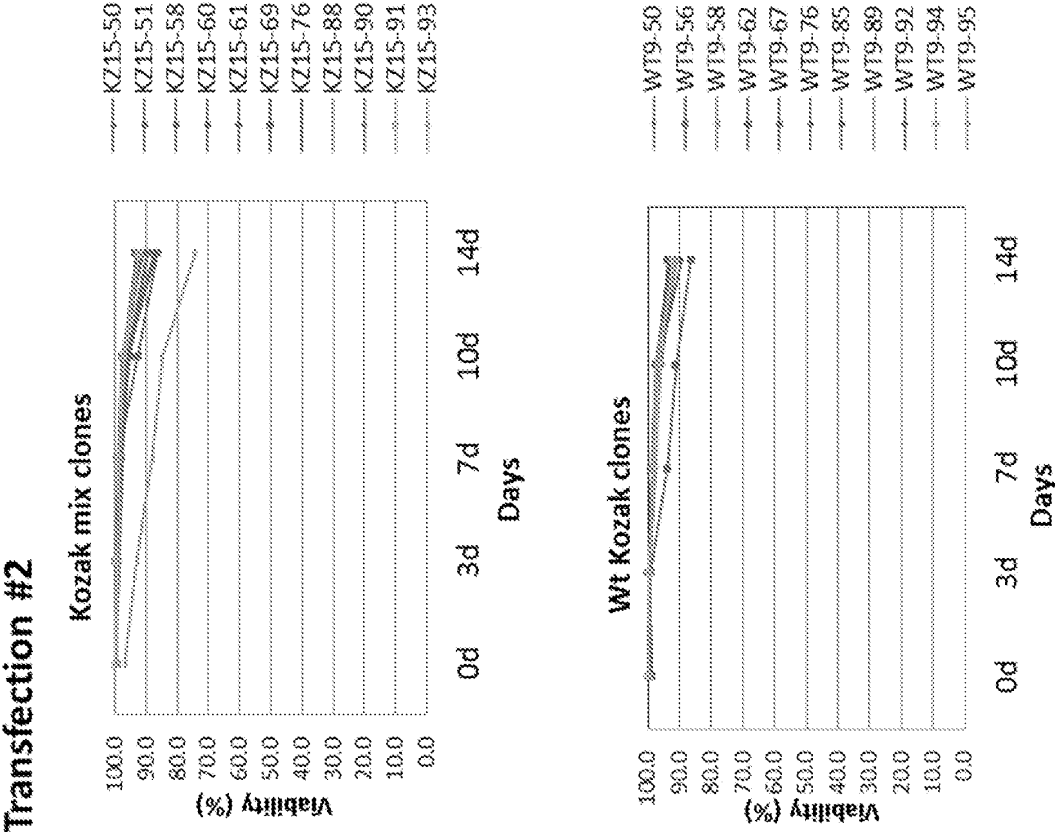


FIG. 16A

Transfection #1

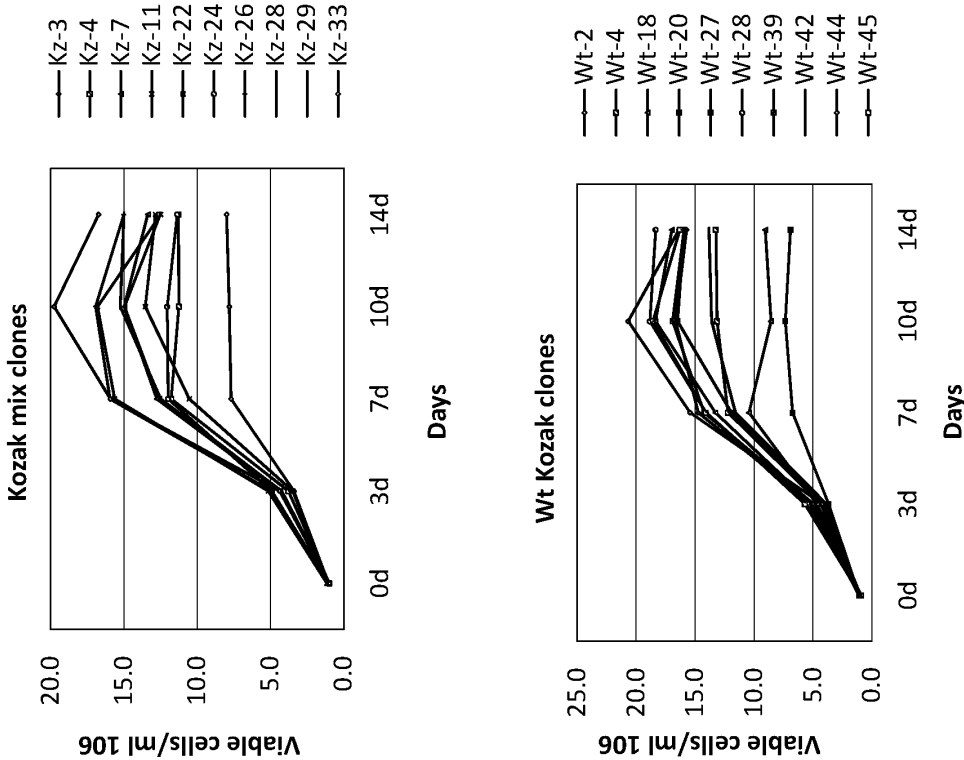


FIG. 16B

Transfection #2

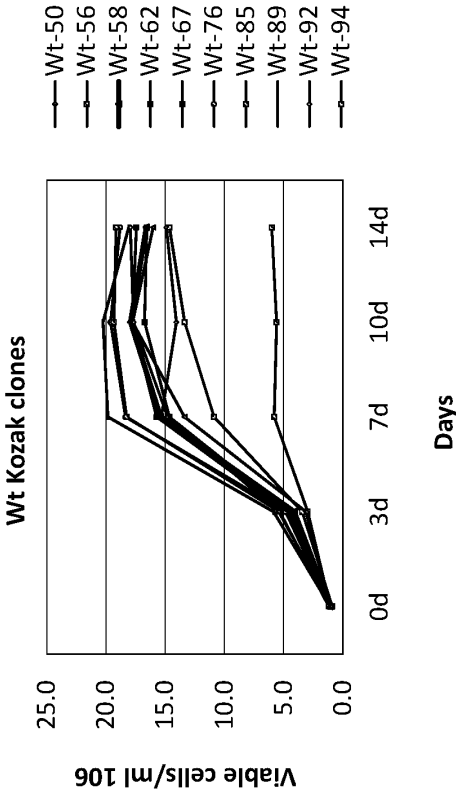
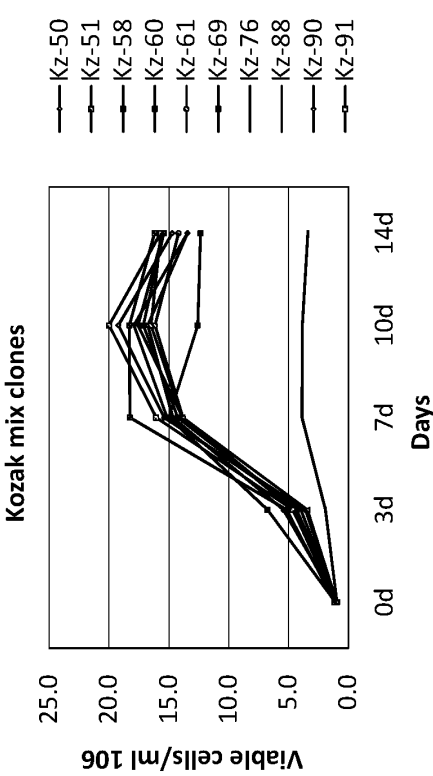


FIG. 17

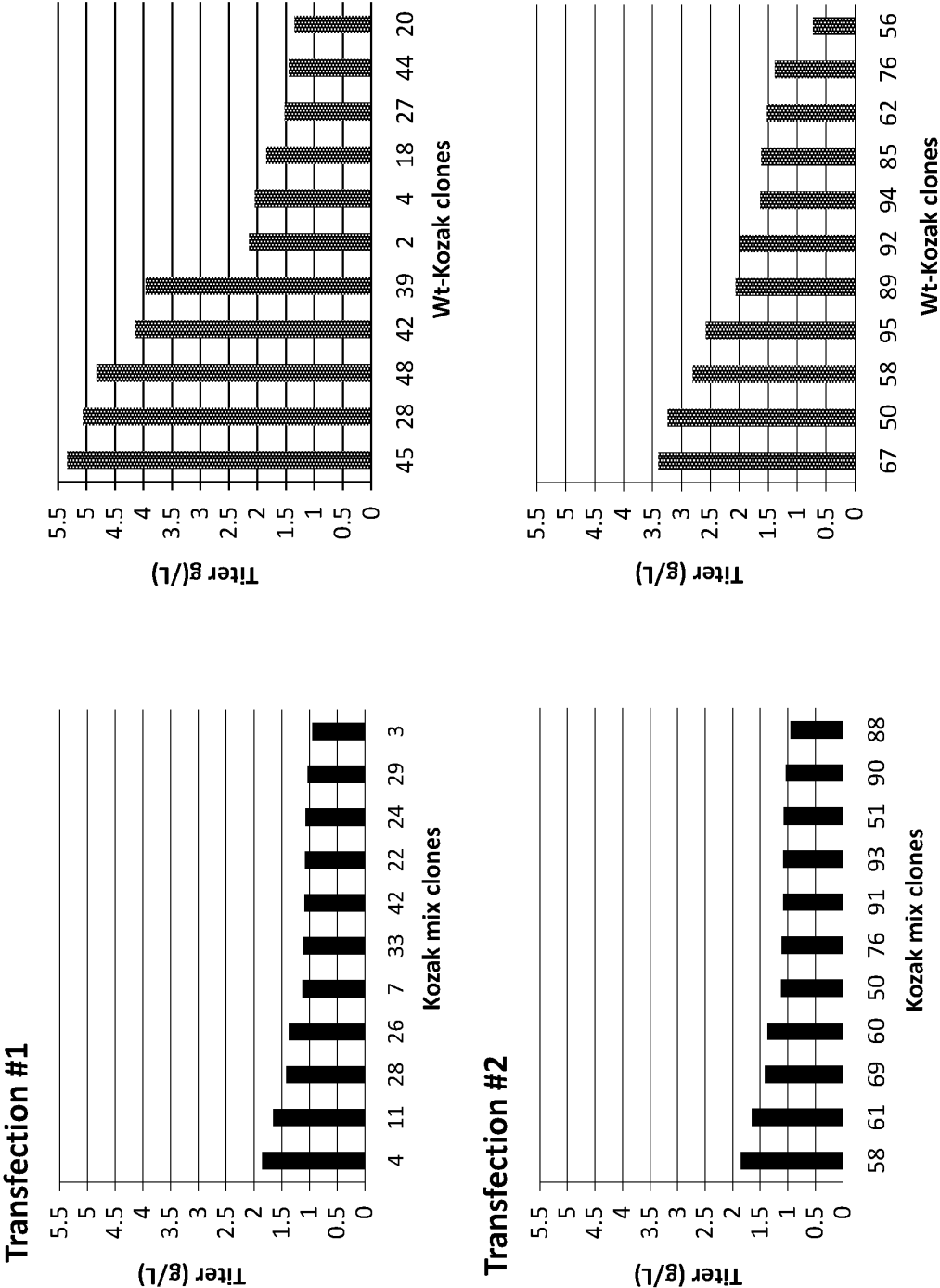
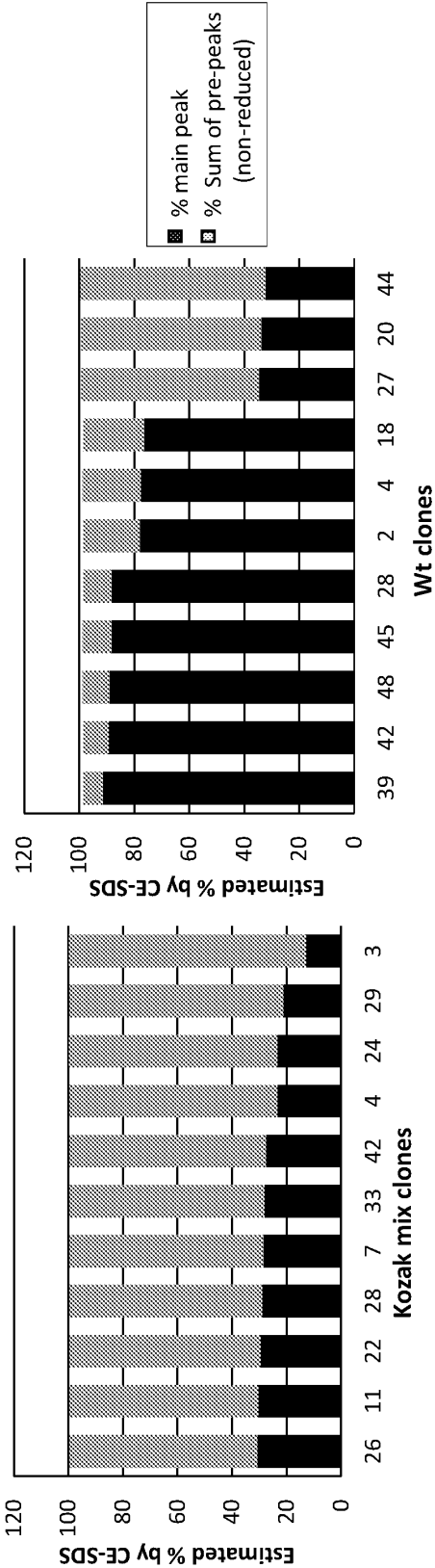


FIG. 18
Transfection #1



Transfection #2

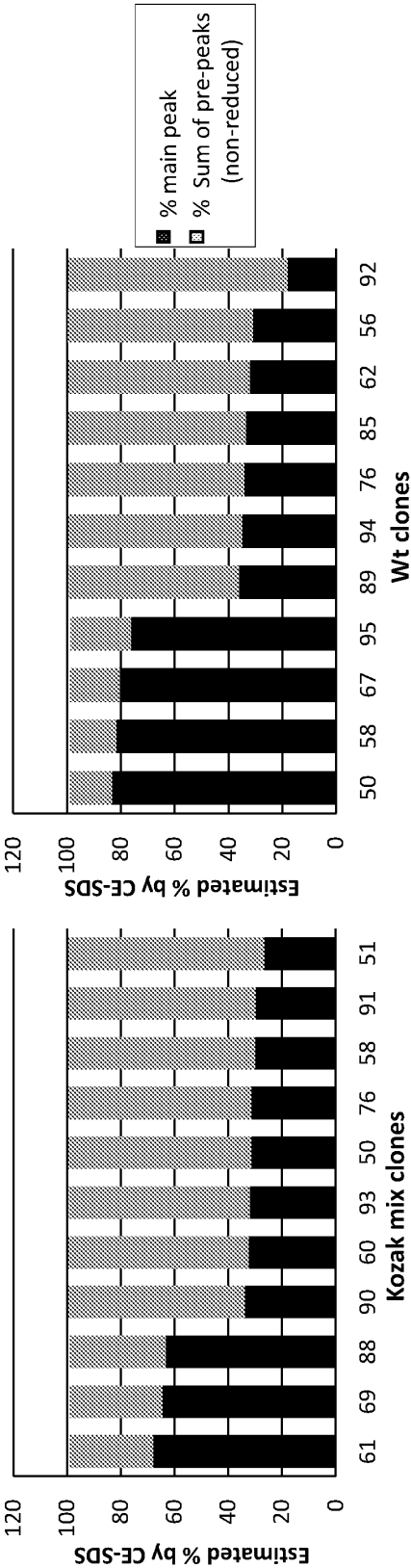


FIG. 19A

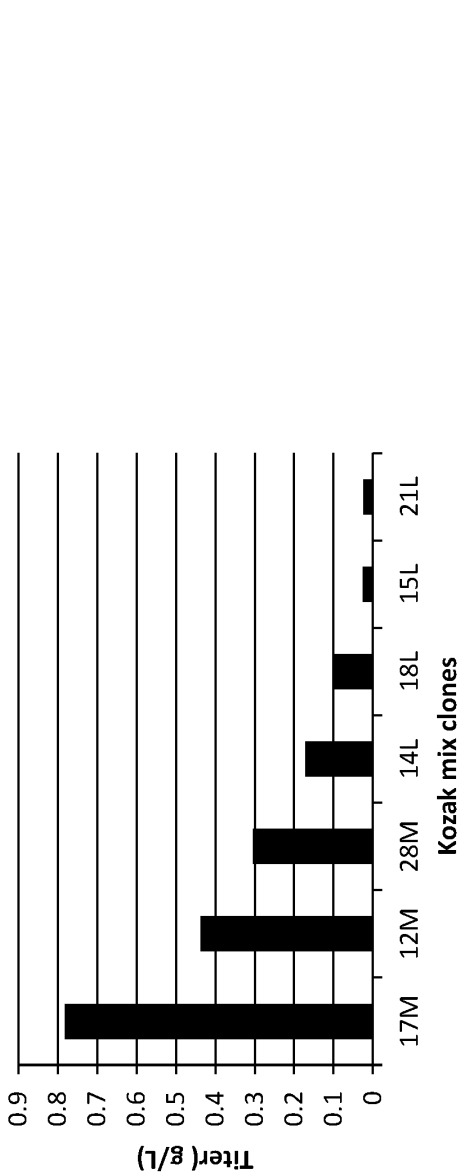


FIG. 19B

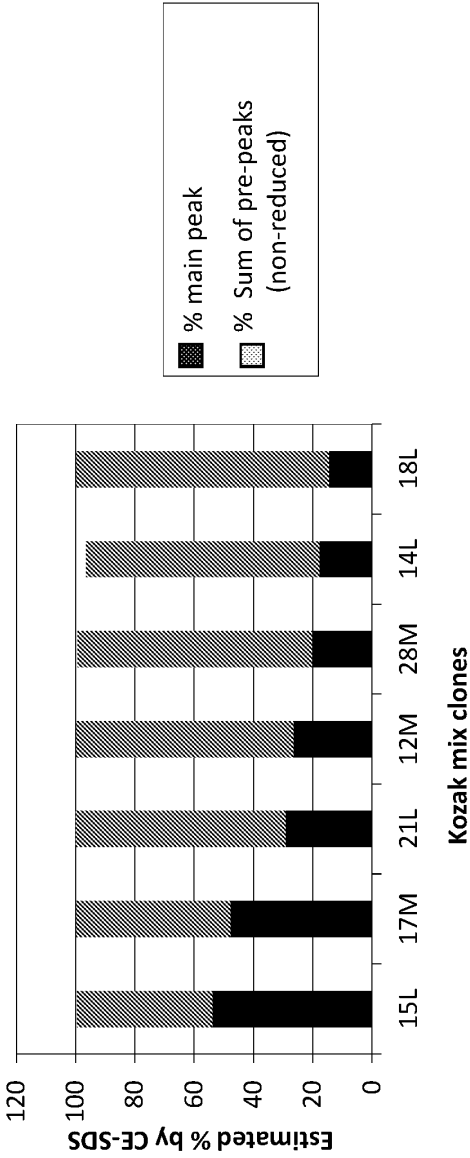
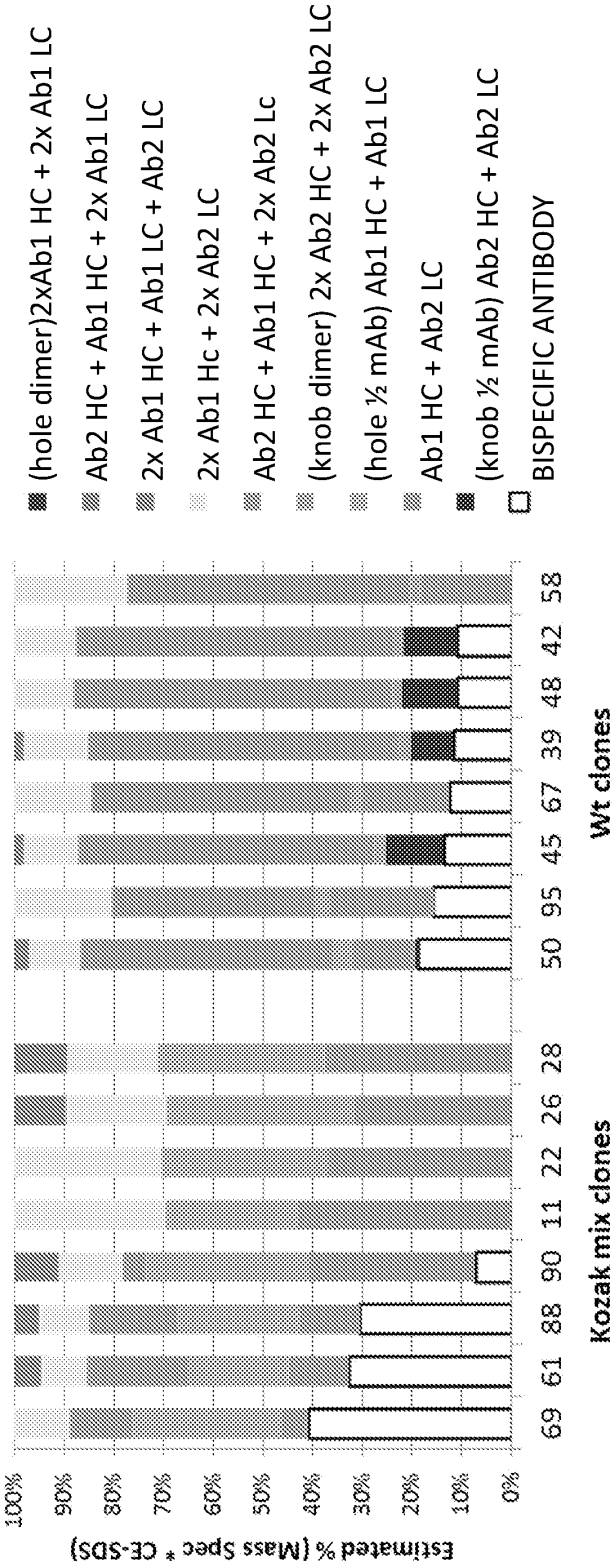


FIG. 20A



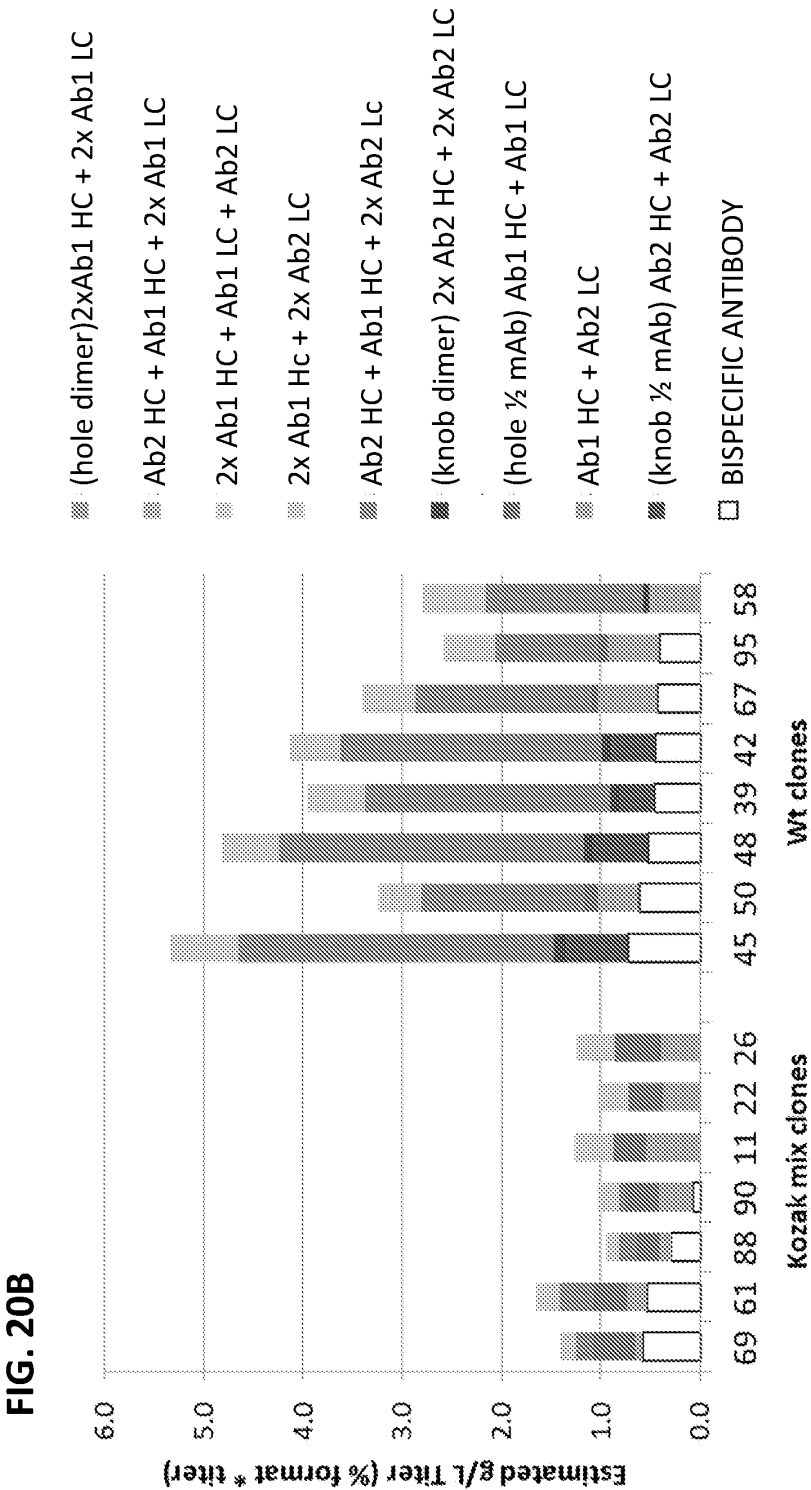


FIG. 21A

	Kozak sequence variant			
	Ab1		Ab2	
Kozak mix clone	HC	LC	HC	LC
69	Kz.228	Kz.228	Kz.228	Wt
61	Kz.228	Wt	Kz.228	Kz.228
88	Kz.228	Wt	Kz.228	Kz.228
90	Kz.228	Wt	Kz.148	Wt
26	Kz.228	Kz.228	Kz.135	Kz.228
11	Kz.228	Wt		Kz.228
22	Wt	Kz.228	Kz.135	Wt
28	Wt	Wt	Kz.3	Wt

Kozak variant	Translational strength
Kz.228	1.3
Wt	1
Kz.148	0.75
Kz.135	0.5
Kz.3	0.3

FIG. 21B

Transfection #1	Kozak sequence variant				Transfection #2	Kozak sequence variant			
	Ab1	Ab2	Ab1	Ab2		Ab1	Ab2	Ab1	Ab2
Kozak mix clone	HC	LC	HC	LC	Kozak mix clone	HC	LC	HC	LC
26	Kz.228	Kz.228	Kz.135	Kz.228	61	Kz.228	Wt	Kz.228	Kz.228
11	Kz.228	Wt		Kz.228	69	Kz.228	Kz.228	Kz.228	Wt
22	Wt	Kz.228	Kz.135	Wt	88	Kz.228	Wt	Kz.228	Kz.228
28	Wt	Wt	Kz.3	Wt	90	Kz.228	Wt	Kz.148	Wt
7	Kz.228	Wt	Kz.135	Wt	60	Wt	Kz.135		Wt
33	Kz.228	Kz.228	Kz.135	Kz.228	93	Kz.228	Wt	Kz.148	Kz.228
42	Kz.228	Kz.228	Kz.135	Kz.228	50	Wt	Kz.228	Kz.148	Wt
4	Wt	Wt	Kz.148	Wt	76	Kz.228	Wt	Kz.148	Kz.228
24			Kz.3	Kz.148	58	Kz.3	Kz.3	Kz.3	Wt
29	Wt		Kz.148	Kz.148	91	Wt	Kz.228	Kz.135	Kz.228
3			Kz.135		51	Kz.228	Wt	Kz.3	Kz.228

FIG. 21C

Kozak mix clone	Kozak sequence variant			
	Ab1		Ab2	
	HC	LC	HC	LC
15L	Wt	Kz.135	Kz.135	Kz.228
17M	Kz.135	Kz.135	Wt	Kz.228
21L	Kz.3		Kz.3	Kz.148
12M	Wt	Kz.228	Kz.135	Wt
28M	Kz.148	Kz.148	Kz.148	Kz.3
14L	Kz.228	Kz.135	Kz.135	Kz.148
18L	Kz.135	Wt	Kz.3	Wt

Kozak variant	Translational strength
Kz.228	1.3
Wt	1
Kz.148	0.75
Kz.135	0.5
Kz.3	0.3

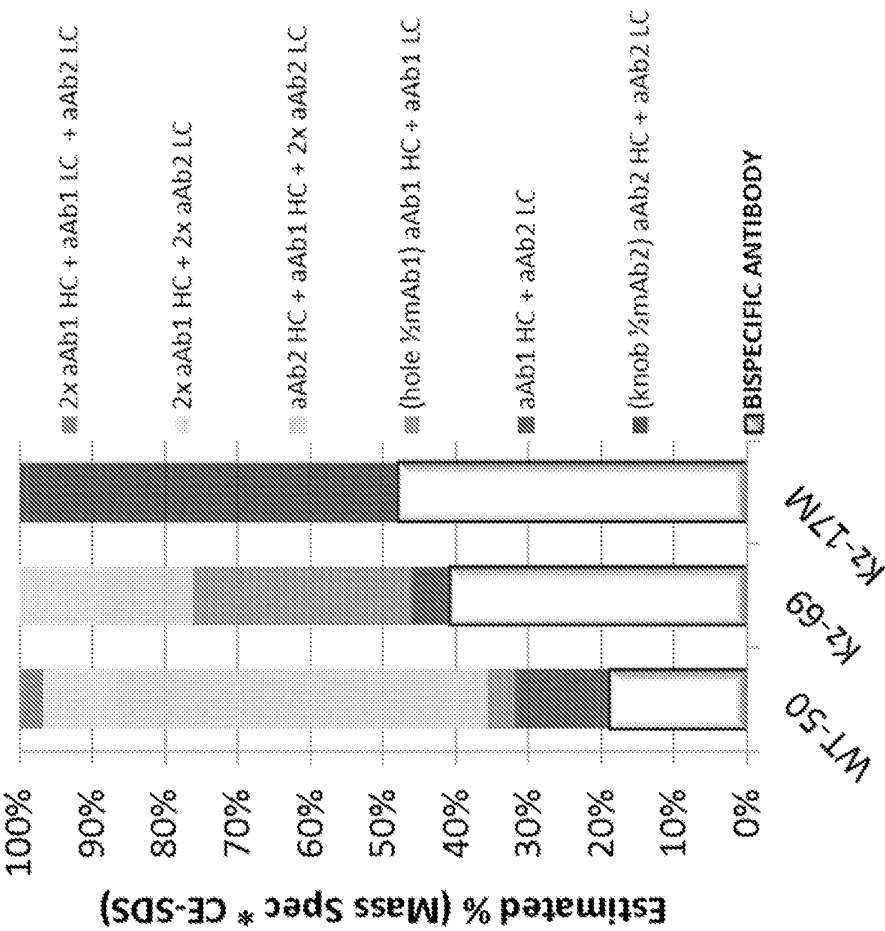


FIG. 22A

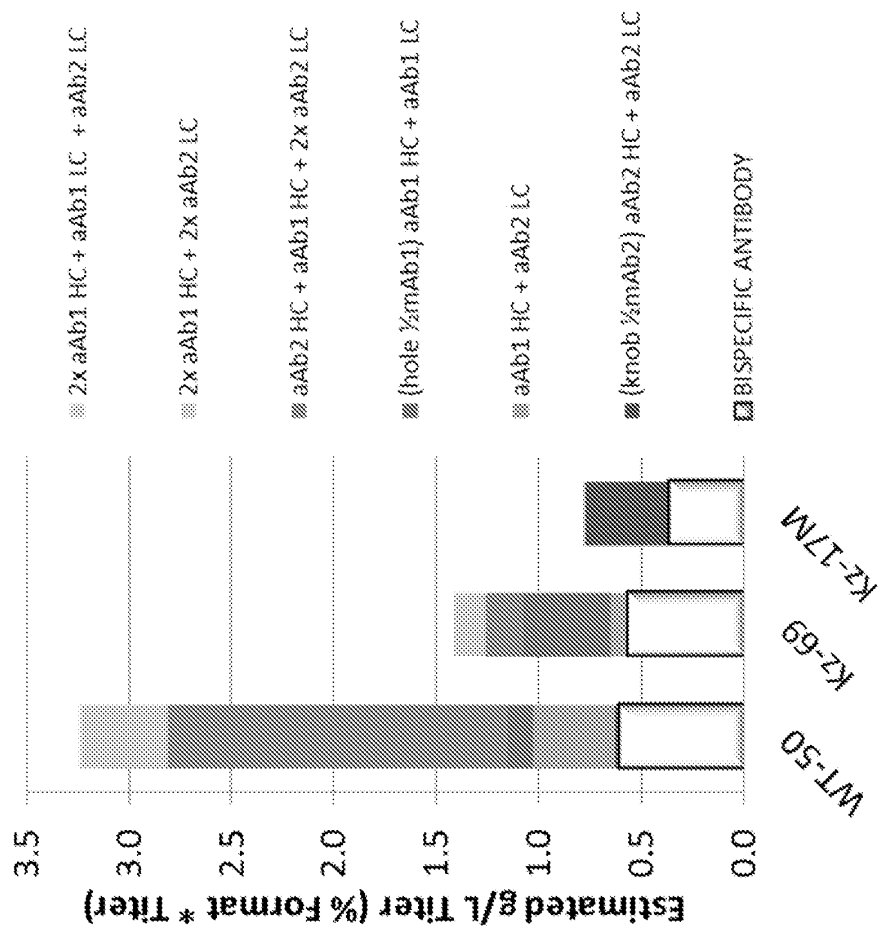


FIG. 22B

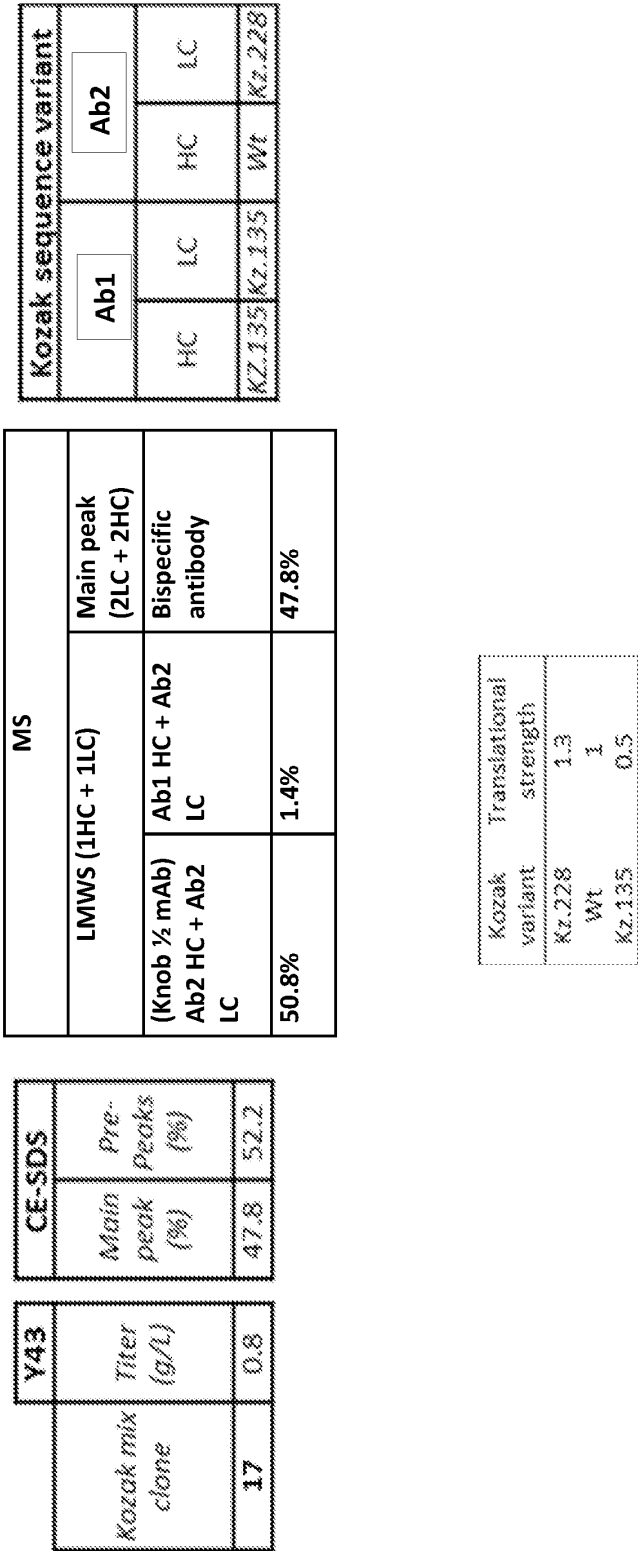


FIG. 22C

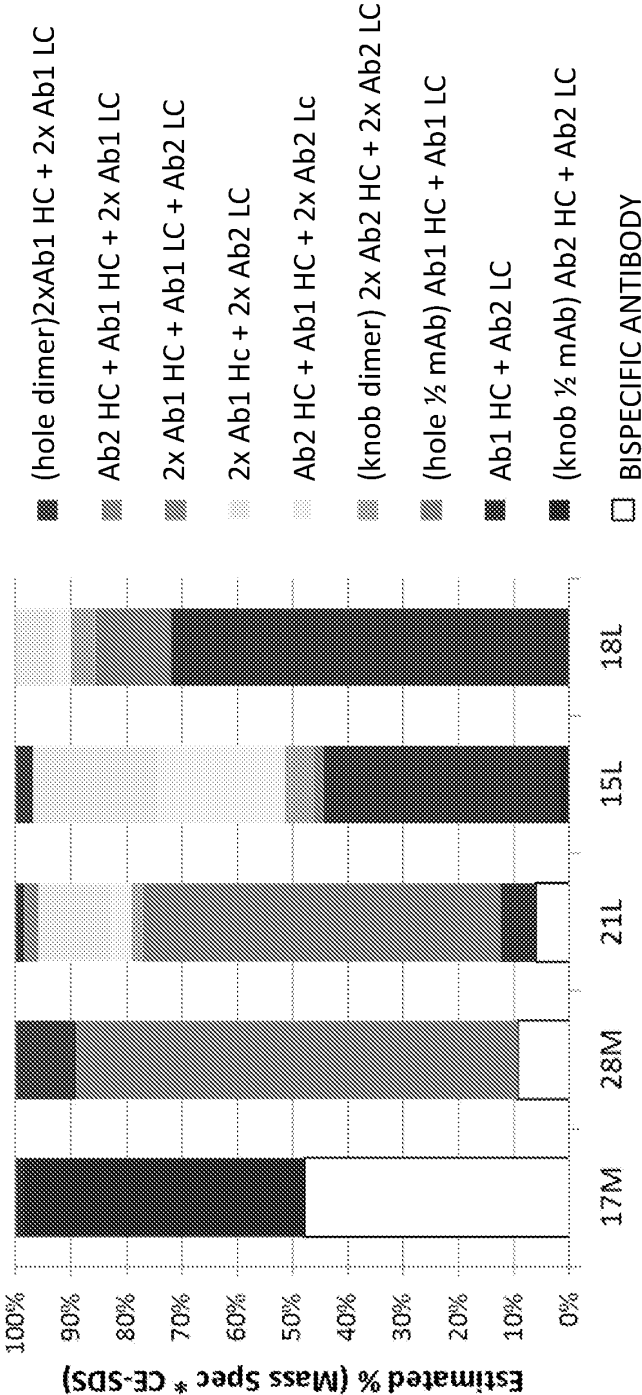


FIG. 22D